EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DIE CHRONISCHE INHALATION VON ZIGARETTENRAUCH BEIM SYRISCHEN GOLDHAMSTER

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EXPERIMENTAL RESEARCH ON CHRONIC INHALATION OF CIGARETTE SMOKE IN THE SYRIAN GOLDHAMSTER

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When we (DONTENWILL and MOHR 1961 a, 1961 b, as well as DONTENWILL and Associates 1962) were able to establish for the first time that Syrian Goldhamsters show a conspicuous organotropism for the carcinogenic effect of nitrosamins in their respiratory tracts, and the experiments of SAFFIOTTI (literature see 1969, 1970) indicated that after influence of aromatic hydrocarbons, growths form in the respiratory tract of the hamsters comparable to the tumors in man, it became obvious to solve the question whether goldhamsters are also suitable for the testing of other known or suspected carcinogens.

Numerous to some extent fundamental experiments by HEROLD and DUNHAM (1962), LASKIN and Assoc. (1970), MOHR (1970) as well as MONTESANO and Assoc. (1970) have meanwhile demonstrated that the goldhamster is particularly suitable for the testing of the effect of carcinogens which upon inhalation can become effective in the area of the respiratory tract.

In our first smoke-treatment experiments with goldhamsters (DONTENWILL and MOHR 1962 a) we copied the known testing techniques by ESSENBERG (1952). However it became evident that a sufficient smoke intake does not take place in smoke-treatment experiments in which the animals are housed in larger chambers. In later experiments (DONTENWILL and WIEBECKE 1966) we reported on test results whereby a stronger inhalation through higher dosage with improved technique was possible. We observed thereby tissue changes in the upper respiratory tract which we compared at that time to precanceroses. Since 1965 through a series of experiments on smoke intake (DONTENWILL and Assoc. 1966, 1967) we obtained the toxicity of CO and nicotine and total smoke (RECKZEH and Assoc. 1969), the effect of smoke-treatment on body weight, blood-count (RECKZEH and DONTENWILL 1970), breath frequency and breath volume (RUECKER and DONTENWILL 1967) as well as through determination of the absorption rate of smoke particles in the upper respiratory tract, numerous informations on the processes in smoke-treatment experiments. Through a series of comparative experiments we developed a smoke-treatment technique which makes a maximal tolerable smoke-exposure of test animals possible. The thereby inhaled smoke dose was kept so high that it rested directly under the limit of the acute toxicity of the total smoke, respectively the most important smoke substances like CO and nicotine. We reported in detail on the preliminary research necessary for the development of this new smoke-treatment test at a conference of the International Union against Cancer at Lausanne 1959 (see TANNENBAUM 1970). We were thereby able to demonstrate that the absorption of smoke particles in the upper respiratory tract, like cavity of the nose but also oral cavity, rests under 50 % (DONTENWILL and Assoc. 1971) and were able to establish through our experiments with radioactively marked space as well as experiments with color substances (CHEVALIER and DONTENWILL 1972) which we offered as Aerosol, that on the basis

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of physical data in nasal breathing a large part of the particles is deposited in the area of the larynx. Relative to the unit of area of the larynx, the particle quantity deposited there amounts to a multiple of the deposited quantity of smoke particles in the lung per unit of area (see Comparison of Doses III/4). These experiments enabled us to explain why in the preliminary test (pilot test) changes appeared exclusively in the area of the larynx. This pilot test on which we reported at Lausanne 1969 (see Tannenbaum 1970), Gatlinburg (DONTENWILL 1970) and at a conference of the American Health Foundation in New York 1970, demonstrated for the first time, that in maximally dosed smoke-treatment a comparative testing of the biological effect on the respiratory tract is possible, and confirmed that the methodology makes a comparative testing of various smoke qualities possible. This pilot test could not give information on the dose/effect correlation since the number of test animals in the individual dosage groups was too small.

In the following we are reporting on a smoke-treatment experiment for which the test arrangement, methodology and dosage was largely based on the experiences from the pilot test. This smoke-treatment test was intended to give information on the effect of the full smoke of standard and modified cigarettes in the respiratory tract, and thus go beyond the limited possibilities of the Epicutant test in which only the particle-phase of the smoke can be examined. At the same time it was intended to show whether the carcinogenic or cocarcinogenic effect of smoke deserves a greater significance and to determine in which ways a reduction of the biological effect of the inhaled smoke is possible.

Since the evaluation of dose/effect correlation in the testing of weak carcinogenic substances depends to a considerable extent on the survival period (BROCK and (SCHNEIDER 1971), it seemed necessary to report in the test results all observed organ and tissue changes since only in that way a quantitative evaluation of the effect, respectively an estimation of the dose/effect correlation is possible.

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II. Material and Methodology:

Test Animals: The animals used in the chronic smoke-treatment tests were female and male Syrian Goldhamsters which were obtained from Zucca's Hamstery, Vineland, N.J., USA. The upon their arrival five-week old female animals had an average weight of 56.9 g (series I - II) and 59.9 g (group I2 - I8 A), and the male animals of the same age an average weight 58.2 g (K - II) and 57.7 g (I2 - I8 A). The animals of the groups I2 - I8 A were delivered and entered into the experiment 6 months after the groups K - II.

After a three-week adaptation and observation period in the quarantine station, the animals were distributed to the smoke-treatment laboratories. The hamsters of all groups were in the beginning housed in pairs in Makrolon cages type II and were set up single between the 38th and 50th week. The 600 hamsters of group K (control) were kept in pairs in Makrolon cages type II throughout the entire duration of the experiment.

At the outset of the smoke-treatment tests the animals were 8 weeks old; at that time the females had an average weight of 80.4, respectively 84.2 g, the males of 81.2, respectively 80.9 g.

Upon their arrival the hamsters were already infested with tapeworms (Hymenolepis fraterna). Against the tapeworm infestation the hamsters were given during the quarantine period one time Yomesan® * (70mg/kg) with the probang. A second and third dose were given I month, respectively 6 months later. The preparation was

* Yomesan : (N-(2'-chlorine-4'-nitro-phenol)-5-chlorsalicylamid).
Manufacturer: Tropon-werke Koeln.

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fed at that time. Six weeks after test begin a pinworm infestation was found in a share of the hamsters. Against that all animals received twice at intervals of 6 months for 6 days in each instance an admixture of 0.25 % Tasnon (R) * to their drinking water.

The keeping of the animals took place in the "closed system" under Spf-conditions. Details on the keeping of test animals and test animal set-up see DONTENWILL and REICKZEH (1968) and REICKZEH and DONTENWILL (1969).

During the entire length of the test the animals were fed exclusively with "ssniff H". * Food and water were offered ad libitum.

Treatment:

Chart I offers a complete survey over the tested cigarette types, the smoke-treatment and additional treatment with carcinogens as well as the test animal groups.

Smoke-Treatment:

The chronic smoke-treatment of the hamsters took place five times a week (Monday - Friday) with the smoke-treatment device Type Hamburg II(DONTENWILL and Assoc. 1966, DONTENWILL 1970). In contrast to former experiments, through the modification of the smoke-treatment chamber, it was possible to smoke-expose 10 animals at a time in one smoke tube. On the model of the customary standard conditions (puff-volume 35 ml, puff-length 2 sec., puff-frequency 1 x per minute) 30 cigarettes per smoke disc were smoked at a time. The type of tested cigarettes, respectively the treatment method as well as the smoking rhythm are listed schematically in chart I. During a single

**Tasnon : (Piperazinhexahydrate), Manufacturer: Bayer, Leverkusen.

***Manufacturer: Intermast Gesellschaft, Bockum-Hoevel.

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smoke-treatment of 7 - 10 min. in the smoke-treatment device - depending on the smoking period of the used cigarette type - the animals were offered the smoke of 30 cigarettes. In order to guarantee that the animals were supplied with smoke interval-free during the individual smoking periods, the cigarettes were smoked with a constant puff number. An average of 23 mm butt-length was to be maintained thereby. The cigarettes were smoked with the following puff numbers: E_2=10, EN= 9, EG_I=7, EG_3= 6, A= 10, D= 11, K =10 and Z = 8. If individual loads smoked distinctly faster or slower, then these puff numbers were exceeded or curtailed by one puff in order to maintain the butt-length. Contingent upon the construction of the machine the animals were offered air containing about 40 % of the main-stream smoke (see Comparison of Doses, p.). During repeated smoke-exposure of the animals on one test day, a pause of 3 hours was maintained in each instance between the individual smoke-treatments (test planning see also DONTENWILL 1970).

For analytical purposes the cigarettes were smoked under conditions described in the cited report (DONTENWILL and Assoc. 1972) and the corresponding analyses were carried out. The results are compiled in chart 2 a+b .

For the animals treated with the gas-phase of the smoke, a fiberglass filter was installed between the smoke chamber of 35 ml size and the smoke-exposition chamber of 558 ml size.

Test Cigarettes: The cigarettes for the tests were manufactured on present-day cigarette machines according to technical standard. The tobacco sheets were produced from the tobacco mixture supplied by us. In the following description of the sheets we give the information supplied to us by the manufacturer.

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The tobacco mixture was - with one exception - uniform for all test cigarettes and was the equivalent of a blended mixture of the following shares:

55% Virginia	40% US-Virginia
	10% Rhodesian Virginia
	5% other Virginia
35% Orient	18% Greek Orient
	8% Turkish Orient
	9% other Orient
10% Burley	5% US-Burley
	5% other Burley

From this mixture were manufactured the standard cigarettes E_2, the cigarette EN, the filter cigarettes A, D and K, the tobacco sheets for the cigarettes EG_I and EG_3. The cigarettes EG_3 and EN received an additive of 8% $NaNO_3$ relative to the tobacco. In manufacturing the sheets a process was used which is briefly described in the following:

Tobacco Sheet EG_I and EG_3

The sheet was manufactured after a process developed by the Gerlach Co. (DAS 1044695, DAS 1033119, DBP 1256133, DBP I 137988). The tobacco meal was pulped with a mixture of methylene chloride and methanol and mixed with methylcellulose and 1.3-butylenglycol. The mixture was spread on a steel band and dried. The sheet contained in the finishing 13% methylcellulose (OCh_3 - content 30%) and 6.7% 1.3-butylenglycol relative to the dry sheet.

The area weight for sheet EG_I was set at 70 - 80 g/m^2.

In the manufacturing of the sheet for the cigarettes EG_3, 8% $NaNO_3$, relative to the wet weight of the tobacco, was added. In addition this sheet contained 12.2 % methylcellulose and 6.2 % 1.3-butylenglycol. The area weight for the conditioned sheet rests near 73 g/m^2.

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For the filter cigarettes A, D. and K, 17 mm long filter rods with a diameter of 8.03 mm were used. The rods were supplied by the Filtrona Co. (Reinbek near Hamburg). The filter materials were as follows:

A acetate filter 2.I/48000 den, puff resistance : ca. 60 mm Ws

D cellulose filter type Dico/Myria, puff resistance : ca. 40 mm Ws

K charcoal filter type die-pressed Tempo with a charcoal content of 3I0 - 340 mg (=coco-nut shell charcoal - grain size o.5 - I.5 mm - active surface $\geq$ I000 m^2 - binding agent polyethylene), puff resistance: ca. 45 mm Ws

The only cigarette not manufactured from the above listed tobacco mixture was the type Z, the mixture of which was as follows:

Burley		I5 %	
of that	Brazil/Malawi		I0
	Domestic		5
USA-Maryland		6 %	
Java		6 %	
Dark Tobaccos			
(cigar grade)		I9 %	
of that	Domingo		I0
	Manila		5
	Brazil/Colombia		4
Other dark tobaccos		54 %	
of that	Brazil		I6
	Paraguay		I3
	Argentina		I0
	Europe		I5

All cigarettes had a diameter of 8.I mm and a length of 85 mm. The cigarette

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paper had a porosity of 25 ml/cm^2. The cut-width was 0.7 mm. If not otherwise mentioned the cigarettes contained as their only additive 2 % diethyleneglycol as a moisturizing agent.

Treatment of the Test Animals with 9.IO-Dimethyl-I.2-Benzanthracene (DMBA), Diethylnitrosamin (DENA) and Asbestos.

DMBA: In an earlier paper (DONTENWILL and Assoc. I970) we had reported about experimental tests on the effect of initial doses of DMBA on the respiratory tract of the Syrian goldhamster. The hamsters (group I and 2)pre-treated with DMBA in the present experiment received IO days before the beginning of the smoke-treatment, respectively upon test begin, a one-time intratracheal application of 500 γ DMBA in physiological salt solution which contained I % carboxymethylcellulose. The animals which died in the first IO days after the intratracheal treatment were replaced.

DENA: The preliminary treatment with nitrosamins in smoke-treatment experiments proved unsuitable since already minimal doses before they produce changes in the lungs will create tracheal papillomas which largely lead to a closing of the tracheal opening (DONTENWILL I964). Since tracheal papillomas upon initial treatment with small doses of nitrosamin will occur already after a few months, the number of test animals for the chronic smoke-treatment is reduced on account of the rising mortality. WEHNER and Assoc. (I97I) were able to confirm this observation (DONTENWILL 1964). They observed the highest mortality among smoke-exposed animals in the test group additionally treated with nitrosamin. This mortality was almost sevenfold.

We therefore chose a "subsequent treatment" in the 52nd and 53rd week after begin of the chronic smoke-treatment (group I8) with a nitrosamin quantity resting barely above the minimal dose. As"controls" (without smoke-treatment - group I8 A) only so

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many animals treated with nitrosamin were used as were still alive in test group I8 at that time. The animals of both groups received within two weeks (52nd and 53rd week) 4 s.c. injections of a 0.25 % solution of DENA in physiological salt solution = 4 x I mg/I00 g body-weight.

Asbestos: We used blue Cape Asbestos from the waste-fume filter of an asbestos processing plant. The fiber length was set at 50 - 60 µ with normed sieves and microscopically controlled. Subsequently a I % suspension in a I % solution of carboxymethylcellulose in physiological salt solution was made. The suspension was constantly shaken with a whirlmix (CENCO Co.). The animals received 0.05 ml of the suspension intratracheally injected after the method described by SAFFIOTTI and Assoc. (I968). This dosage was chosen because higher dosages as discovered in preliminary tests will lead to extensive inflammable changes which would give reason to expect too great a reduction in the survival period.

Pathological-Anatomical Tests:

An autopsy after the customary dissection method was undertaken on the spontaneously deceased animals and the animals killed at quarter-year intervals of control group K starting with the 6th month.

Deviating from the custormary technique, the throat organs with base of the tongue, larynx, trachea and lungs were totally removed. After the larynx was cut off, the lungs were prepared according to the fixation method described by CHEVALIER (I97I). This guarantees a vastly constant fixation of the lungs equivalent to the anatomical conditions at the outset on which a quantitative evaluation of parenchymal changes (emphysema etc.) is possible in a considerably more precise manner.

The heads were fixed in toto in order to avoid damage of the nasal respiratory tract.

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Vertical cuts were made through four levels of the head, and in each instance at least three cuts of each level were examined.

Experience had shown that a complete seizing of macroscopically not visible changes, particularly in the area of the respiratory tract is only possible if as many cuts as possible of the individual organs are examined. Beginning with the 25th test week we made gradational sections particularly of the organs of the respiratory tract. As a rule we examined in all test animals beginning with the 26th test week ca. I2o gradational profiles of the larynx, 50 of the trachea, 6 of the lung, I2 of the suprarenal gland and as a rule 3 of the remaining organs. Generally we evaluated hematoxylin-eosin colorations, in particular instances we made different special colorations (e.g. in the tumors of the lymphatic tissue) (Congo red coloration, Berlin blue reaction, Elastica van Gieson and Weigert fibrin coloration, Giemsa, PAS, silvering after Goemori, chloracetatesterase proof).

Statistical Evaluation Method:

For the statistical evaluation of the tests numerous quantitative and qualitiative findings for each test animal were available. Since the test animals were kept under largely homogeneous conditions, over-coincidental differences in these measured values can be attributed to the influence of the treatment. Since the evaluation was made with the help of electronic computer systems, all findings had to be put on voucher sheets first and transferred from there to computer cards. Examples of these voucher sheets for quantitative and qualitative findings are shown in ill. I and 2. In the qualitative findings a I was entered if the particular finding was present in an animal (e.g. pharynx inflammation), otherwise we entered a 0, respectively left the field blank.

For the statistical evaluation of the quantitative values, the customary statistical quantities like average value, standard deviation, standard deviation of the average,

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range etc. were calculated for each test group and each sex. The differences between the average values of different test groups and sexes were tested with the help of variance analyses and summarizing also with a multivariate analysis. Simultaneously with this multivariate analysis, the correlations between the weights of the organs and the weights of the animals ~~was~~ were calculated.

Of particular interest is the question of the age dependency of the findings. This question was statistically examined with a regression calculation. In addition the linear regression to age was calculated and tested for all body and organ weights.

In order to be able to examine a possible connection between the organ weights and the body weight, the relative organ weights (relative to 100 g body weight) were calculated for each animal, and the average values of these relative weights were established for all groups. The differences between the groups were also tested here with variance analyses. In addition to that, a multiple regression to age and body weight was calculated for all organ weights. Thus it was possible to analyze in summary the influence of these two variables on the weight of the organs.

The frequency was counted for the qualitative pathological findings in each group and for each sex. Differences between these frequencies in different groups were examined in an χ^2-test if such an examination seemed mathematically expedient and significant.

In order to be able to recognize a possible dependency of weight developments and lenght of life on different pathological findings, the average of the weight changes as well as that of the test length was additionally calculated and compared to the total average for those animals which showed the pathological finding of interest. Significant differences between these two averages indicated a dependency of the quantitative measured values from the qualitative findings.

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Analogus to that, the frequency of certain pathological findings, particularly of tumors, was also counted in different age groups and the age dependency of these findings was thereby examined.

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III. Test Results

Survival Period: The comparative observation of the survival period of the test and control animals was expected to give clues on the total toxicity of the smoke substances. This is facilitated in the present test since we are able to compare different control groups (the untreated controls group 3 and the placebo group 8) with test groups to whom we administered varied doses of smoke. The average survival periods are compiled in chart 3. The differences between the different test groups were statistically examined with F-tests. The results of these tests (for all quantitative measured values) are presented in summary in chart 4.

For the survival period we obtained significant differences ($p < 5\ \%$) for the males between the smoke-exposed groups 4, 5 and 6, and between the smoke-exposed groups (4, 5 and 6) and the control group (group 3) respectively the placebo group (group 8) extreme/significant differences ($p < 1\ \%$) in the sense of a reduced life expectancy of the smoke-exposed animals.

For the females no significant differences could be found ($p > 5\ \%$) between the three different doses of smoke-exposure (group 4, 5 and 6). Likewide no difference could be proven between the placebo group 8 and the smoke-exposed groups 4, 5 and 6. However the average survival period for the females was also significantly higher in control group 3 with 57.34 weeks than in the smoke-exposed groups 4, 5 and 6 ($p < 1\ \%$). The differences in the average survival periods between control group 3 and the placebo group 8 could be ascertained statistically high ($p < 1\ \%$) for the males as well as the females (see Variance Analyses Chart 4).

It can be established in summary that the smoke-treatment in comparison between smoke-exposed animals and untreated controls causes a reduction of the survival period. Beyond that, a dose-dependent shortening of the survival period could be ascertained for the male animals.

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A significant difference in the survival period is also found between the untreated controls of group 3 and the animals of group 8 treated only with fresh air in the smoking apparatus. This indicates that the manipulation in the smoking apparatus already leads to a shortening of the survival period.

Changes of Body Weight between Test Begin and Test End

In chart 5 are compiled for the test groups the average change of body weight at test end relative to the average weight of the groups at test begin as well as the standard deviation and the standard deviation of the average (graph, ill. 3).

The F-test yielded for the males as well as the females extreme/significant differences between the three smoke-exposed groups 4, 5 and 6 and between the smoke-exposed groups and control group 3 respectively placebo group 8 ($p < 1\%$).

In order to be able to examine statistically the dependency of the end weight from the survival period, a linear regression between the two variables was calculated for each group and the significance of this regression was tested, chart 6. In the chart the regression coefficient b and the test measure F are listed for each instance. b states for how many gramms per week the end-weight changes. F is a measure for the reliability of this coefficient (see below).

We obtained for the control groups 3 an extreme significant correlation between the end-weight and the survival period ($p < 1\%$); for the groups 4 and 6 a significant age dependency could not be established, for group 5 only a weak significant one. The correlation between weight change and survival period is positive in groups 3 and 5; which means, with increasing survival period the body weight increases too. In group 6 however the correlation was negative for both sexes. Here the body weight decreased with increasing survival period. In the remaining groups no unequivocal correlation between weight change and survival period could generally be established.

Dependency of the Weights of the Organs from the Survival Period

For all organ weights a regression to the survival period was calculated (chart 7 - 1)

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and tested for its significance against 0. The regression coefficient b and the pertinent F-value are stated in the charts. The regression coefficient b expresses the average change of the organ weight per one week change of the survival period. When b is Zero the organ weight in question does not change in the average with the survival period; which means, there is no correlation between the organ weight and the survival period (in the range of the observed survival periods). In order to be able to test this hypothesis b = 0 statistically, the value F was also stated in the chart. This value F is the quotient of b^2 and the variance of b. If the hypothesis b = 0 is correct, the F-values are smaller than 4 with a probability of 95 % and with a probability of 99 % smaller than 7. If the calculated F-values exceed these limits, we have a case of a significant respectively extreme significant correlation between organ weight and survival period.

The correlation between the heart weight and the survival period is extreme significant for the females in the groups I, 2, 3, 5 and I0. For the males the correlation is only in group I4 extreme significant. An effect of the smoke-treatment can not be recognized. The significance of the correlation should be largely dependent on how the observed survival periods are dispersed and whether varying organic development phases can also be seized thereby. This is indicated by the high significance in group K in which many animals were killed prematurely.

The correlation between the liver weight and the survival period is for the males as well as the females in almost all the groups extreme significant. However an effect of the smoke-treatment can not be recognized.

The age dependency of the liver weights should be primarily attributable to the increasing frequency of cysts in longer survival periods.

The correlation between the kidney weight and the survival period is however in the females only in groups I - I0 extreme significant (except for group 3 and 6) and in the males in groups 3, 5 and I6. A correlation to the smoke-treatment is also not found here.

In the weight of the suprarenal gland an extreme significant correlation to the survival period exists for the females only in the groups 5 and 6. In the males however the correlation in these groups is not significant. It is therefore

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questionable whether the age dependency of the weight of the suprarenal gland in group 5 and 6 is correlated to the smoke-treatment.

The testicle weights show in most groups a significant negative correlation to the survival period. Accordingly the testicle weight decreases with increasing age. This should be attributable to the frequency of atrophies increasing with age.

In addition to this regression analysis, the correlation coefficients between the quantitative measured values were also calculated. The calculation was done for all groups, separated in males and females, whereby the varied group average was taken into consideration (correlation within the group). The correlation coefficients are entered in chart I2.

It became evident that only a very weak correlation exists between the weight difference and the survival period (in females positive, in males negative) which is statistically not significant. However, the correlation between heart weight and survival period, liver weight and survival period and kidney weight and survival period is significant for the males as well as the females. The correlation between suprarenal gland weight and survival period is for the females not, for the males weak significant. In addition there is a relative high negative correlation between the testicle weight and the survival period. In the observation of the dependency of the organ weights on the survival period an unequivocal effect of the smoke-treatment could not be established.

Organ Weights

The average organ weights for the different test groups and the sexes are presented in charts I3 - I7. The differences were statistically examined here also with a variance analysis and an F-test.

Extreme/significant, sex-specific differences for all groups were found only in the heart weight and the kidney weight ($p < 1\%$). In the liver weights there was no difference at all between males and females, and in the suprarenal gland weights

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the differences were only weak/significant ($p < 5\ \%$). Conspicuous in the liver weights was the great standard deviation which was approximately as large as the average value in several groups. This corresponds to a variability coefficient of I. Another similarly high variability coefficient was observed only in the gonad weights. This high standard deviation of the liver weight is certainly caused by the frequent appearance of liquid-containing cysts of the bile-duct.

In the males differences between the group average values for the heart weight, liver weight, kidney weight and the gonad weight were statistically ascertained (probability of error $< I\ \%$). No significant differences for the suprarenal gland weight were ascertained between the groups. In the females the differences were weak/significant for the liver weight while extreme/significant differences were ascertained for the heart weight, kidney weight and the weight of the suprarenal gland.

A dose-dependency, that is, differences between the groups 4, 5 and 6 were ascertained in the males only for the kidney weight ($p < I\ \%$). In the females the differences between these three groups were also extreme/significant for the heart weight.

If only the groups 3 as well as placebo group 8 are compared to the smoke-exposed groups 4, 5 and 6, a high significance was provable in the males only for the kidney weight. In the females this difference between the groups 3, 8 and 4, 5 and 6 was also extreme/significant for the heart weight; the weights in question were in group 6 with the highest smoke dose considerably lower than in the control group 3 and the placebo group 8. In the relative heart and kidney weights (relative to I00 g body weight) this however holds true only for the females. In the males the average relative heart and kidney weight is in group 6 even higher than in group 3. This can be explained by the fact that the differences in the organ weights between group 6 and group 3 were lower than the corresponding differences of the body weights.

A particularly large difference between the smoke-exposed group 6 and the control

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group 3 respectively the placebo group 8 was found in the heart weights of the females. The relative decrease in the average heart, kidney and liver weights of the smoke-exposed test groups and the lacking relative decrease of the average weight of the suprarenal gland stands in a, through experience in human pathology, understandable relation to the body weight. GIESE and HORSTEBROCK (I962) have compiled numerous observations for quantitative lack of food which show the same pattern (chart I8). While with the decrease in body weight an almost parallel decrease of the heart, liver and kidney weight is observed in most instances, the suprarenal gland shows a different behaviour;relative weight increases of the suprarenal gland are to some extent observed during malnutrition respectively weight loss. The findings show that the weight changes observed by us are a case of an indirect, not specific effect of the smoke-treatment on the weight decrease which is effected by the smoke-treatment. It was conspicuous in the testicle weights that significant differences existed neither between the three dose groups 4, 5 and 6 nor between the control group, the placebo group and the smoke-exposed groups. However, the testicle weights in the groups I and 2 as well as in the groups 7, IO and I5 were significantly lower than in the remaining groups. The animals of group 8 had the highest average testicle weight. However, this weight was not significantly higher than that of group 3 but differed extreme/significant from that of group 7 as well as 2, I, IO and I5. In so the decrease of the testicle weight in group I and 2, the effect of DMBA could be discussed. In total, the comparisons between the groups do not demonstrate a definite influence of the smoke-treatment on the average testicle weight.

Correlation between Organ Weight and End-Weight and End-Weight of the Animal - relative Organ Weight

The organ weights are always negatively correlated with the end-weight respectively the weight difference. A particularly high negative correlation exists between the liver weight and the end-weight and the kidney weight and the end-weight. However,

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the correlation between the suprarenal gland weight and the end-weight is not significant in the female and only weak significant in the male. The correlation between the testicle weight and the end-weight is stronly negative.

Of the remaining correlations it is worth mentioning only that the kidney weight is significantly positively correlated with the heart weight as well as with the liver weight. The suprarenal gland weight shows another significant correlation only to the kidney weight.

Chart I9 lists the average values of the relative organ weights, relative to I00 g animal weight, for all groups separated by sex. The conclusions drawn already in the examination of the correlation between the organ weights and the body weight are thereby confirmed.

For the heart in the females we found only in group 6 a remarkably low relative weight of less than I g per I00 g animal weight. This difference is weak significant compared to the relative heart weight of group 3.

In the males however, there is no difference between group 6 and group 3. In both groups the relative heart weight is comparatively low.

For the relative liver weight in the females no significant difference between the different groups could be found. In the males the difference was weak significant. This significance can be explained primarily through the relatively low weight in group 8 with 5.67 g per I00 g animal weight. The relative liver weight is also comparatively low in the groups 4, 5 and 6. However, it is significantly higher in group 3 with 8.08 g per I00 g animal weight. But this should not be attributable to an influence of the smoke-treatment. Otherwise we should have to expect a high relative liver weight also in placebo group 8.

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkf0000

For the kidney weights it is particularly striking that in the females the relative kidney weight of group 6 with I.9 g per I00 g animal weight is the lowest. On the other hand, there is no difference between the relative kidney weights of the groups 3, 4, 5 and 8. Here too, we can not establish a definite influence of the smoke-treatment. In the males there is no significant difference between the relative kidney weights in the smoke-exposed groups 4, 5 and 6 and the control group 3 respectively the placebo group 8.

For the relative suprarenal gland weights it is striking that in the females they are significantly higher in the smoke-exposed groups 4, 5 and 6 than in control group 3 or placebo group 8. In the males however, no difference shows between the smoke-exposed and the not smoke-treated groups. Whether therefore the result for the females is attributable to an influence of the smoke-treatment remains questionable.

For the testicle weights too, there is no significant difference to be found between the smoke-exposed groups 4, 5 and 6 and control group 3 respectively placebo group 8. However, the relative testicle weight in group K with I.62 g is significantly higher than in the remaining groups. Furthermore, it is striking that the weights in the smoke-exposed groups 4, 5 and 6 as well as in control group 3 and group 8 are higher than the relative weights in most of the remaining groups. Merely in the groups I6, I7, I8 and I8 a similarly high relative testicle weights as in the smoke-exposed groups 4, 5 and 6 were found.

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Source: https://www.industrydocuments.ucsf.edu/docs/xmkf0000

III. 2 a Diseases of the Respiratory Tract:

(Metaplasiae, Tumors, Inflammations etc.)

Spontaneous tumors of the respiratory tract are extremely rare in the goldhamster as emphasized already earlier at symposia (Perugia - I966, Gatlinburg - I969) by numerous researchers.

We (DONTENWILL and WIEBECKE, I966) had formerly assumed on the basis of observations on a large stock of animals that the frequency of so-called spontaneous lung adenomatoses amounts to about I/I000. FORTNER (I958) saw among 620 hamsters 3 bronchus carcinomas, KIRKMAN (I962) among 7.200 hamsters 3 adenomatoses, 2 adenomas, 2 adenocarcinomas, DUNHAM and HERROLD (I962) among 360 hamsters I adenocarcinoma, LEE and Assoc. (I963) among 37 hamsters I adenomatosis. CHESTERMAN (I964) describes the observation of benign tumors of the lung (quantity not stated). From the descriptions on hand it is not definitely recognizable whether the described adenomatoses and adenocarcinomas of the hamster lung are cases of a pseudoadenomatosis which we (CHEVALIER and DONTENWILL, I97I) count among the "adenomatoid lesions" and separate from the tumors.

While spontaneous tumors of the larynx have so far not been described and spontaneous tumors of the lung are extremely rare, many authors have reported about induced tumors of the respiratory tract. In the beginning we reported on observations of tumors of the respiratory tract after intratracheal application of Benzpyrene and after varying treatment with Nitrosamins. In addition it should be mentioned that DELLA PORTA and Assoc. (I958) and GROSS and Assoc. (I965) succeeded in producing carcinomas of the larynx, the trachea and the lung through multiple intratracheal application of DMBA. According to the experiences which we made with the examinations of the deposit of radioactively marked smoke components (DONTENWILL and Assoc. I97I), we had to count the nasal respiratory ducts as well as the oral respiratory ducts among the respiratory tract since both ducts , even if to a varying degree, can be used in the process of inhalation.

Inflammable changes occurring in the area of the oral cavity were relatively frequently provable in smoke-exposed as well as control animals. In comparison between smoke-exposed animals and controls (3 and 8) a significant influence of the smoke-treatment could not be established (chart 20) ($X^2 = 9.67$ with 4 latitudinal degrees;* ($p \approx 5\ \%$)). Inflammable changes in the area of the nasal cavity were more frequently provable in individual groups among the smoke-exposed animals, particularly among

*latitudinal degrees = Freiheitsgrade, abrev. FG maintained throughout the text. Tr. note.

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Source: https://www.industrydocuments.ucsf.edu/docs/mlbk0000

the male animals, (e.g. group 5, 6 and I4), (chart 20). In the males we also found highly significant differences between the smoke-exposed groups (particularly group 6) and the control and placebo group ($X^2 = 18.2$; FG = 4, (P<I %).

Unequivocally atypical epithelium metaplasiae of the mucous membrane of the mouth were provable neither in the control animals nor also in the smoke-exposed animals, except for one animal treated with the gas-phase of the smoke. Papillary tumors starting from the pavement epithelium of the mucous membranes of the mouth, we were able to observe only in one each of the animals treated with DMBA of group I and 2 as well as in one animal treated with DENA of group I8A. Papillary tumors in the area of the palate were found predominantly in the animals treated with DMBA, occasionally also in untreated or smoke-exposed animals (ill. 4). There were also sporadic appearances of infiltratively growing carcinomas of the oral cavity, to some extent starting from the cheek-pouch (ill. 5 and 6). A correlation to the smoke-treatment is not present.

Charts 2I and 22 show the distribution of frequency in the individual groups.

In the nasal cavity no unequivocally metaplastic changes of the mucous membrane were found as we have described them formerly after smoke-treatment (DONTENWILL 1970).

The inflammable changes of the nasal cavity occurred with approximately the same frequency in treated and untreated animals. They were alwayd accompanying symptoms of a phlegmonous change in the soft parts of the head. We calculated the frequency differences between the groups 3, 4, 5, 6 and 8 (chart 20). The differences between this frequency were examined with an X^2- Test. The test yielded for the females no significant differences ($X^2 = 4.86$ with 4 FG; (P> 5 %)). In contrast, the differences in the males were highly significant ($X^2 = 18.17$ with 4 FG; (P< I %)). These high differences in the males are exclusively attributable to the high value in group 5,

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Source: https://www.industrydocuments.ucsf.edu/docs/xwb0000a

in which I5 animals, that is I8.75 % showed inflammations of the nasal cavity. Since however in group 6 with the highest smoke dose only 5 animals, that is 6.25 %, showed inflammations of the nasal cavity, it is questionable , whether the high share in group 6 may be attributed to an effect of the smoke-treatment.

In individual animals we observed adenomas in the area of the nasal cavities which apparently proceeded from the mucus glands. It was a case of tubular adenomas which do not show an infiltrative growth process, (chart 2I and Ill. 7). Since the adenomas also appear in the control groups K and 3, a correlation to the treatment is not assumed. Malignant tumors of the nasal cavity were not observed.
(Two and one half lines illegible in German Text. Tr.n.)

... difference between males and females is 895.0 with I latitudinal degree. This corresponds to an error probability of considerably less than I %. In the females the X^2-value for the difference between the groups 3, 4, 5 and 6 amounts to I6.9 with 3 latitudinal degrees. This difference is significant at $P < I$ %. The frequency of the concrements is significantly higher in the control group 3 with 56 % than in the smoke-exposed groups 4, 5 and 6 with maximally 4I % in group 4. Consequently, the frequency of the concrements is reduced by the smoke-treatment. These generally layered stones fill the gland clearances in parts completely and lead to the atrophy of the epithelium. They are predominantly provable in female animals. No unequivocal statements can be made on the make-up of the red-brown concrements on account of the colorations which were undertaken (HE, iron, (Berlin blue) Ziehl-Neelsen, Kossa, PAS, bleaching with H_2O_2).

In individual animals we found benign tubular to papillary adenomas of Harder's Gland (chart 23 and ill. 9).

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkf0000

In the pharynx papillary tumors proceeding from the pavement epithelium were provable predominantly in the animals pretreated with DMBA (chart 24), except for one beginning carcinoma in one animal of group I (ill. II)they did not show any infiltrative growth. They were only sporadically observed in untreated and smoke-exposed animals. A correlation to the smoke-treatment is not present.

Papillary tumors of the trachea (chart 24) as DONTENWILL and MOHR (I96I c), HERROLD and DUNHAM (I962, I963), MONTESANO, SAFFIOTTI and SHUBIK (I970), ALTHOFF, WILSON and MOHR (I97I) and others described them predominantly after treatment with diethylnitrosamin (DENA), we found relatively frequently in the animals treated with DENA of the groups I8 and I8A, but also sporadically in the animals treated with DMBA of t groups I and 2. The structure of papillary not infiltratively growing tumors induced through DENA treatment ressembles the morbid growths formerly described by us and compared to the pavement epithellium ressembling tumors of the bladder (ill. I2). After the more recent examinations on semi-thin profiles by ALTHOFF, WILSON and MOHR (I97I) it can be assumed that these papillary growths of the trachea are morbid growths which are not made up of metaplastic pavement epithelia but of cilia-carrying columnar epithelia, respectively of mucus producing cells. The contrasting of the two groups I and 2 as well as I8 and I8A shows that the smoke-treatment in the animals pretreated with nitrosamin has led to an increase of the papillary tracheal tumors. Since no tracheal tumors occurred through smoke-treatment alone even in high dosage, the effect visible here is to be essentially understood as a promoter effect of the smoke respectively a cocarcinogenic effect.

As already in our preliminary examinations (DONTENWILL, I970), the larynx region of the hamster shows also in the present experiment in all treated groups the most severe changes. As earlier, we divided the changes into 6 stages as they are presented schematically in ill. I3,and in the classification we have used that scheme (chart 25)

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Source: https://www.industrydocuments.ucsf.edu/docs/mkkb0000

as the basis for the division of stages. The "Atlas of Tumor Pathology" published by Ash and Assoc. (1964) served as the basis for this division of stages.

The individual stages (see ill. 13, 14 a-m) were based on the following criteria (chart 26) as characteristics. The frequency of the described larynx changes is compiled in chart 27. They shall be discussed in detail subsequently to the description of morphologically deviating findings in the larynx.

Besides these characteristic changes a papillary tumor was found in one instance in the larynx under the pattern of a fibroepithelioma in group 10 with considerably stronger development of the stroma than in the typical papillomas.

Of the described larynx changes (stage I - 6), morphologically well delimitable were papillomas which were traceable in the lower sections of the larynx only in the animals treated with DENA (group 18 and 18A). In group 18 two females and 19 males and in group 18A 5 females and 10 males had larynx papillomas. The sex difference is significant.

These papillomas showed in their structure a vast similarily to the papillomas of the trachea observed after nitrosamin treatment, in respect to the tissue structure as well as the total structure. (ill. 15 a and b).

In the comparative observation of the larynx changes we settled first of all the question whether leukoplakias of the larynx respectively larynx tumors were traceable in the altogether 960 not-treated animals (which means, not smoke-exposed and not treated with carcinogens). We found only in one animal of group K and in no animal of the groups 3 and 8 changes in the sense of stage 2, in none of the animals the stages 3 - 6. Only stage I was traceable, to some extent combined with inflammable processes. Also in the animals of group 7 treated only with the gas-phase, only

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Source: https://www.industrydocuments.ucsf.edu/docs/mkk0000

pachydermias (stage I) were found in 9 animals, which means, no larynx changes of stages 2 - 6. Larynx changes of the stages 2 - 6 were also not observed in the animals of group I3 and group I8A. In the evaluation of the different effect of the treatment within the individual groups in comparison to the controls, we included in our calculation the time of the appearance of the changes in the larynx as well as the degree of severity of the changes. The frequency distribution of the stages 2 - 6 was evaluated in the same way as the age of the animals at the time of the examination respectively at the time of the proof of the described larynx changes (chart 27 - 37). In viewing the charts, it must be taken into consideration that often several stages appeared side by side respectively simultaneously in the same animal.

Correlation between Age (Survival Period) and Stage of the Leukoplakia of the Larynx

In order to be able to analyse this correlation, we counted for each treatment group separated in male and female, the frequency of the different leukoplakia stages in 5 different age groups (0-25, 26-50, 5I-75, 76-I00, >I00 weeks). The results are compiled in the charts 28 - 37. It is to be observed thereby that one animal could show several stages simultaneously. The total number of stages can therefore be higher than the number of animals within a certain age group. The percentages listed with the discovered numbers relate in each instance to the number of animals within the age group in question. This number is listed in the second column.

The charts show that in all smoke-exposed groups, with exception of group 7 treated with the gas-phase of the smoke, there exists a pronounced dependency between the age and the leukoplakia stage; high leukoplakia stages occur predominantly with advanced age; that is, all the animals of this age group often have one leukoplakia within stage 3 - 5 respectively carcinomas (stage 6). For the quantification of these dependencies, Pearson's Contingency Measure was calculated for the mentioned

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groups. The males and females were thereby taken together in one group eventhough different age distributions do indeed exist in the sexes, but the dependency ratios are not influenced by them. These contingency tables and the appertaining contingency measures are listed in charts 38 - 42. These contingency measures show a high dependency between age and leukoplakia stage.

It must be observed that these contingency tables are relative to the distribution of leukoplakia cases and not the number of animals. Accordingly, the total number is not a constant quantity but a coincidental quantity. Moreover, the numbers in the different stages are statistically dependent since to some extent they refer to the same animals. The quantity X^2 is therefore not X^2 distributed.

In order to test the correlation between age groups and leukoplakia stages, ARMITAGE (I97I), suggested to compare the frequency of the stages with a Poisson distribution whose parameter is estimated from all age distributions. This test resulted in all contingency tables in a highly significant dependency.

It is remarkable that the distribution of leukoplakia stages can be divided into three different types.

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The first type is present in the smoke-exposed groups 5 and 6. In this type the stages 3 and 5 occur with approximately the same frequency, and approximately 25 - 30 % of the animals are afflicted by it. The age distribution of stage 5 is shifted more to the higher age groups in comparison to the distribution of stage 3.

The second type is present in the groups 4, 9, I0 and II. In this type stage 3 predominates but it also includes only 25 - 30 % of the animals. The high stages, particularly stage 5 and 6 are relatively rare.

The third type is present in the groups I2 and I4 - I8. It is characterized by the great frequency of stage 3 (65 - 78 %) which occurs predominantly in the age groups 5I - 75 weeks. It is also striking in this distribution that stage 5 occurs relatively frequently and stage 4 relatively rarely. In these groups stage 4 often seems to be

Source: https://www.industrydocuments.ucsf.edu/docs/mlkb0000

"skipped". This is corroborated by the fact that the age distribution of stages 3 and 5 runs similarly while the distribution of stage 4 is shifted somewhat more to the younger age groups.

The dose/effect correlation is particularly unequivocal in the comparison of groups I, 2, 4, 5, 6 with the controls (groups 3, 8 and K). While the controls showed almost no larynx changes of the stages 2 - 6 (only one case of stage 2 in group K), the groups 2, 4, 5, 6, I showed in approximately this sequence changes in the area of the larynx to an increasing extent. Particularly the degree of severity (of stages 2 - 6) was thereby increasing with increased length of application and increasing dose, whereby the smoke-exposed animals with additional DMBA-treatment showed the strongest findings (group I).

Pronounced was the considerable difference in the comparison between the effect of DMBA alone as well as DMBA and smoke or smoke alone, this becomes particularly evident in the female animals and in the total number of animals.

This not simply additively explainable increase of the effect through initial treatment with DMBA, particularly in the female animals, argues for an influence of cocarcinogenic factors (besides the carcinogenic factors) of smoke.

The comparison between group 5 and the groups 9, IO and II, that is, between the same-dosed treatment with the standard cigarette and the treatment with the cigarettes containing natrium-nitrate, the sheet containing natrium-nitrate and the sheet alone demonstrates that particularly the larynx changes stage 5 and 6 are reduced in the groups 9, IO and II. This reduction cannot be explained through a varying treatment time. The natrium-nitrate admixture to the tobacco produces therefore, just as in the skin-test (DONTENWILL and others, I972) a strong reduction of the biological effect of the smoke. The fact that, with additional nitrosamin treatment, not an increased but indeed a reduced effect is provable, argues against an additive effect of smoke

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and nitrosamin on the same place in the larynx. Nitrosamin alone is not able in the applied dose to produce morphologically equivalent larynx changes of the stages 2 - 6, as they occur in smoke-exposed animals.

The cigarettes manufctured from tobacco sheets show - similar to the skin-test (DONTENWILL and others, I972) - a distinctly reduced biological effect. In both tests (cigarettes with natrium-nitrate and cigarettes from sheets) an effect is provable which runs approximately parallel to that of the skin-test.

The minor lung changes in group I3 (preliminary treatment with asbestos) show that the asbestos dose which was used, was too low. The comparison between group 5 and I2 shows that almost the same result could be obtained with the same smoke dose in the animals which were taken into the test 6 months later.

A considerable reduction of the biological activity, measured by the frequency of the stages 5 and 6, was also shown by the groups I4 (black cigarette), I5 (cigarette with acetate filter) and I6 (cigarette with Dico-filter). The reduction is somewhat smaller for the cigarette with charcoal filter. The very frequent appearance of stage 3 is conspicuous in these groups. Approximately 70 % of the animals of these groups showed larynx changes in the sense of stage 3.

The fact that in group 7 (treatment with the gas-phase of the smoke) changes of the stages 2 - 6 did not occur, allows us to assume with certainty that the carcinogenic factors are to be sought chiefly in the particle-phase.

In viewing the charts, it becomes furthermore evident , that with a relatively weak influence respectively at the beginning of the treatment, stage 3 predominates in the larynx changes, and that with an increasing degree of severity of the larynx changes and an increasing length of the treatment, stage 3 of the larynx changes decreases while in contrast the stages 4 - 6 increase at the same time. Consequently, it is a case of a, so to speak, step by step malignization of the epithelium whereby

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

it is not necessary that all stages have to be passed through.

The lacking infiltration into the cartiladge of epithelium growths in stage 6, designated as "early invasive carcinoma", is a finding which can be explained by the reaction between tumor growth and cartiladge. Also in earlier examinations (DONTENWILL and MOHR, I96I), we did not see any carcinoma infiltration into the cartiladges of the trachea or the larynx.

The tumor growth spreads almost exclusively between the cartiladge links respectively in the zone of the pars membranacea. In comparative examinations on the ears of mice (DONTENWILL and Associates I972), we were able to show that also in the large tumors of the mouse-ear, produced by Benzpyrene, the cartiladge is almost never attacked by the tumor growth. Apparently, the cartiladge constitutes an extraordinarily resistant barrier against induced tumor growth.

A joint quantitative evaluation of the individual stages of the larynx changes and with that a summarization of these changes per group does not seem to be possible. The present examinations demonstrate solely that stage 3 apparently constitutes a first step towards the stages 5 and 6 and that the stages 5 and 6 are expressions of a stronger tumor-triggering effect. While in our opinion, in comparison to the experiences in man (BUNGELER and DONTENWILL, I954, DONTENWILL, I966), the stages 2, 3 and 4 can be assessed as precanceroses "in the wider meaning", we would like to regard stage 5 as a precancerosis "in the narrower meaning".

Inflammable changes of the larynx were not unequivocally more frequently provable in the smoke-exposed animals as compared to the controls (chart 20) (X^2 = I3.04 with 4 FG (P < I %)). Inflammations and tumor formation show no parallelity.

In relation to the changes observed in the upper respiratory tract area, the number of tumors of the bronchi and the lung are extraordinarily small. Bronchial papillomas (chart 24) as they had been described formerly with nitrosamin treatment and the

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Source: https://www.industrydocuments.ucsf.edu/docs/mkf...

influence of Benzpyrene, we found only sporadically in the animals treated solely with DMBA and in the hamsters of group I8 and I8A treated with nitrosamin (ill. I6 a plus b). The varying localization of DMBA - respectively DENA - induced changes is thereby remarkable as was already found in the comparison of the place of attack of smoke-components and nitrosamin.

In the lung we could prove only one infiltratively growing pavement epithelium carcinoma in one animal treated simultaneously with smoke and DMBA (ill. I7).

In 3 animals (2 males of group 2 and I female of group 5) we saw mucus-producing adenomas (ill. I8) proceeding from the bronchus, as SAFFIOTTI (I970) described them after intratracheal BP-application.

Unequivocal metaplasiae of the bronchial mucous membrane were found relatively rarely. Metaplasiae were found only in group I (2 animals) and 6 (I animal), that is, in animals which were additionally treated with DMBA or smoke-treated with the highest dose. Bronchial epithelium changes,as AUERBACH (I97I) was able to prove them in the chronic smoke-treatment of dogs,were not observed by us.

Lung: Macrophagi, Microliths, "Adenomatoid Lesions".

Of importance is also the frequency distribution of the following lung changes:

I. Macrophagi accumulations in the alveoli with brown, iron-positive pigment which we registered after OTTO (I963) as "smoke cells".
2. Accumulations of pigment-free macrophagi,
3. The appearance of microliths in the alveoli,
4. The frequency of "adenomatoid lesions" of the lung.

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Chart 43 shows that the so-called "smoke cells" (ill. I9) also occur in not smoke-exposed animals and are therefore not to be understood as specific changes, that is, are not actually to be understood as "smoke-cells in the meaning of OTTO, which

Source: https://www.industrydocuments.ucsf.edu/docs/mfk00000

has already been pointed out by CONNING (1969). The frequency of the "smoke cells" is unequivocally higher in the smoke-exposed groups than in the control groups K, 3 and 8 (X^2 = 176.5 with 6 FG (P< 1 %)) in the females and (X^2 = 141.3 with 6 FG (P< 1 %) in the males. It can be assumed that the particle-phase of the smoke is more strongly responsible for that than the gas-phase which becomes particularly evident in the male hamsters in the comparison of the groups 5 and 6 to 7 (X^2 = 38.6 with 2 FG (P< 1

The finding for group I (DMBA and smoke) and 12 (asbestos and smoke) shows that other factors can also influence the frequency of the "smoke cells"(differences between the groups I, 12 and 3 highly significant (X^2 = 144.8 with 2 FG (P< 1 %)).

Pigment-free macrophagi appeared particularly in the animals pretreated with asbestos of the groups 12 and 13 whereby often asbestos particles (ill. 20) and ironpigment deposits became visible.

The distinct increase of pigment-free macrophagi is also found in the animals of group 12 pretreated with asbestos and smoke.

The microliths show the greatest frequency apparently in the animals pretreated with DMBA. The difference to group K is highly significant (X^2 = 81.4 with 2 FG). On the other hand,there is no significant difference between the smoke-exposed groups 4, 5 and 6 and control group 3 as well as placebo group 8 (X^2 = 4.02 with 4 FG). It is possible that microliths are formed after cell damages through the intratracheally applied cancerogen, solved in CMC,which was the cause of microliths formation.

"Adenomatoid Lesions" of the Lung

In chart 43 we compiled the changes in the sense of a pseudoadenomatosis, better the so-called "adenomatoid lesions"which were traceable in the individual groups.

The pseudoadenomatoses or "adenomatoid lesions" belong to the most frequent lung change in the hamster colony which we used for the present experiment and show a very varied range. They are, as we had already earlier mentioned in detail, not to be mistaken

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

for carcinomas of the lung (CHEVALIER and DONTENWILL, 1971). Earlier we dealt in detail with the genesis of the "adenomatoid lesions" of the lung and were able to find that also in the present examinations, the "pseudoadenomatosis" particularly with a simultaneous appearance of inflammable lung changes (bronchopneumonias) was often very strong in its range. Ill. 22 a and b show one diffuse and one focal "pseudoadenomatosis" whereby the cilia-carrying epithelia of the bronchus almost completely line the alveoli and thereby give the misleading impression of adenoma-ressembling changes which are often falsely interpreted as tumors (see CHEVALIER and DONTENWILL, 1971). "Adenomatoid lesions" respectively pseudoadenomatoses were also traceable in the controls as chart 43 indicates. An influence of the treatment on their frequency is statistically only weakly ascertained ($X^2 = 11.67$ with 4 DFG ($P > 1\ \%$)). A dose dependency is however not provable ($P > 5\ \%$).

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Source: https://www.industrydocuments.ucsf.edu/docs/mkk0000

Lung: Thrombi - Infarcts - Vascular Wall Inflammations

After chronic smoke-treatment of dogs HAMMOND, AUERBACH, KIRMAN and GARFINKEL (1970) and others, observed an increased appearance of lung thrombi.

We saw in the lung thrombi as well as inflammable vascular changes often combined with lung vessel thrombi in the sense of a thrombangiitis. Dense inflammable infiltrates (particularly leukocytes and lymphocytes) in the vessel wall and in the vessel vicinity and vascular wall swelling to the point of fibrinoid necrosis were traceable thereby. The findings show a vast similarity to inflammable vascular changes of the coronary arteries and the peripheral arteries.

Chart x plus y offers a survey of the frequency of thrombi and inflammable vascular processes. A hemorrhagic infarct of the lung was observed only in one animal of group

The comparison of the frequencies between the individual groups does not show an increase of the changes in the smoke-exposed animals. In the females the test also yielded differences between the groups 3, 4, 5, 6 and 8, an χ^2 of 4.52 and in the males of 2.44, for 4 latitudinal degrees in each instance. These differences are not significant ($P > 5\ \%$).

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Source: https://www.industrydocuments.ucsf.edu/docs/mkk06000

Lung Emphysema - Pneumonia - Bronchitis

HALMOND and Assoc.(1970) as well as AUERBACH and Assoc. (1970) reported one lung emphysema in the dog after chronic smoke-treatment. Also in other smoke-treatment experiments, for instance, in the rabbit (HOLLAND and Assoc. 1963) the formation of a lung emphysema was reported.

In the hamster, an extraordinarily strong lung emphysema after treatment with papain, was described by several authors (GOLDRING et al. 1968, GOLDRING and Assoc. 1970, GROSS and Assoc. 1968, 1971). The lung emphysema proven in these experiments was as a rule traceable throughout the entire lung and often showed an extreme expansion. In these changes the lung emphysema could be diagnosed as a rule already in simple histological examinations.

Through numerous examinations on man it was possible to demonstrate that the diagnosis lung emphysema can only be ascertained with specific methods on tissue profiles (HARTUNG 1959; OTTO 1968a, b).

As we already mentioned in the beginning, we fixed the lungs in our experiments after the method described by CHEVALIER (1971), in order to be able to proceed from a dilated state of the lung in vivo in the comparative evaluation of the lung changes and particularly in the measuring of the degree of severity of the lung emphysema. All lungs were largely fixed in the same state of dilation which facilitated a comparative evaluation of the condition of the lung alveoli. As the chart shows, an unequivocal emphysema according to criteria used in human pathology could be diagnosed only in a few animals during the microscopic examination of the lungs.

An unequivocal accumulation of the diagnosis emphysema, ill. 23, was not recognizable in the treated animals (chart 45). Since it is not possible with the small animal to carry out the process of emphysema evaluation used today in human pathology on a paper-mounted large surface profile with the aid of a screen-system (OTTO 1970), we attempted to carry out quantitatively comparative measurements through other methods.

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We made comparative measurements on the following groups:

Group K : 10 ♂ and 10 ♀ animals with an age of 9 - 32 weeks (=4. - 27. test week) was compared to

Group 3 : 20 ♂ animals of the 104. - 129. test week and
20 ♀ of the 65. - 93. test week as well as

Group 6 : 20 ♂ animals of the 71. - 111. test week and

Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

20 ♀ animals of the 56. - 87. test week.

The animals of group 6 had been smoke-treated daily with 3 x 30 cigarettes. We selected these three groups in order to compare lungs of healthy, young animals (group K) with lungs of untreated controls (group 3) of a more advanced age and animals which had been smoke-treated with a high dose for a long time.

All lungs were fixed after the initially described method, which means, dilated to the normal volume and subsequently embedded in the usual manner, cut and colored. We were careful to keep a largely constant cut-width and a coloration of even strength. In order to arrive at an evaluation largely independent from the researcher in the assessment of the lungs, we decided for a densiometric process of emphysema measuring (GLAUSER and GLAUSER 1968, LONGFIELD and HENTEL 1969). The measurements were carried out with the microscope-photometer MPV of the Leitz Co. after attachment of a grey-filter.

We measured the transparency in % in a lung area of always the same size. This could be focussed precisely with the help of the available cross-wires and square diaphragms (o.6 x 0. (illegible, Tr.) mm). The examinations were carried out in a darkened room. The zero-value was re-examined before each measurement. In the focussing of the lung sections which were to be measured, we were carefull to make sure that no larger bronchi or blood-vessels rested within the measuring area. This was the reason why the measuring area could not be chosen much larger.

We made 5 respectively Io measurements per lung. Lungs with strongly inflammable changes, atelectases or strong congestion could not be applied in the evaluation. The measurements resulted in the following:

male animals:

	group K	group 3	group 6
number of animals	IO	20	20
number of measurements	IOO	IOO	IOO
transparency			
% average value	80.63	80.62	8I.29

According to the variance analysis the differences between the groups were statistically not significant (P> 5 %).

female animals:

	group K	group 3	group 6
number of animals	IO	20	20
number of measurements	IOO	IOO	IOO
transparency			
% average value	79.38	79.84	79.II

Here too no difference could be found (P> 5 %).

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Source: https://www.industrydocuments.ucsf.edu/docs/phkk0020

The present results demonstrate that a difference between the controls and the chronically smoke-exposed animals was not determinable through a densiometric evaluation. Consequently, the lung emphysema rate was not increased by the smoke-treatment.

For the purpose of controling the densiometric evaluation we used a further method. Since, as mentioned in the beginning, an evaluation by means of large surface profiles was not possible, we tried to do quantitative measuring with a similar method.

The lungs were projected on the table top in medium enlargement (object. A 0.25) by means of a drawing-mirror (Leitz). A screen of I0 mm side length and 2 mm gradation, mounted on the ocular (I2x) made a counting of the lung alveoli possible on drawing-paper. The actual size of the measured zone, that is, the zone delimited by the screen was 0.93 x 0.93 mm. All the alveoli or small bronchi within the range of the screen and projected onto the paper were marked on the paper with an ink-pen. Five different zones were evaluated per lung. The number of the marked cavities was subsequently counted. In contrast to the densiometric method, the profiles of all lungs with two exceptions (one atelectatic lung and one lung with catarrhalic inflammation) could be evaluated.

Since differences between smoke-exposed and not smoke-exposed animals among the male animals with a longer survival period were the most likely to be expected, the lungs of group 3 and 6 were examined. This yielded no significant differences ($P > 5\ \%$).

male animals:

	group 3 controls I04. - I29. week	group 6 smoke-exposed animals 7I. - III. week
number of animals	20	20
number of measurements	I00	I00
alveoli/unit, average value	I76.27	I70.96

The present comparative examinations show that with high-dosed, long-lasting smoke-exposure, an unequivocal increase in lung emphysema in the smoke-exposed animals was not provable with the applied measuring methods.

To supplement the evaluation it must be added that stronger inflammable changes in

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

the area of the bronchi (with exception in the range of bronchopneumonias) were extraordinarily rare in the controls and the treated animals. Chart 45 shows the frequency of bronchopneumonia and of severe bronchitis in the individual test groups. A significant increase of these findings was not provable in the smoke-exposed animals. Whether the maintenance of the test animals as described in the beginning is also responsible for that can not be determined.

It should be mentioned in addition that bronchiectases with transition to the so-called cyst lung were observed only once. Whether their formation constitutes a similar process as in the cysts of the liver and other organs can not be evaluated on the one case.

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Source: https://www.industrydocuments.ucsf.edu/docs/mkk60000

III.
2b Liver

Already in earlier experiments (DONTENWILL and EDER (1959), DONTENWILL and MOHR (1961), we frequently observed single or multiple cysts of the bile-duct, to some extent arranged in conglomerates, in the liver of goldhamsters (ill. 24). As formerly, we distinguished between liver cysts and the so-called cyst-liver in which the entire liver is penetrated by numerous cysts and the extremely enlarged organ shows only remains of parenchyma between the densely settled cysts (ill. 25). The evaluation of the test results shows that the frequency of cysts is larger in the female animals, but that a significant difference can not be proven because of the strong dispersion. A correlation to sex of the cyst-liver is not recognizable. ($X^2 = 3.26$ with 1 FG; ($P > 5\ \%$). An increase in cysts, as we and others had formerly observed in the carcinogen treatment of the liver (LAUBER and DANNENBERG (1956), DONTENWILL and MOHR (1961 a plus b), DONTENWILL (1961), BANHASCH and REISS (1971), RIVIÈRE and OBERMAN (1967), is not provable in the smoke-exposed animals. - Cysts in other organs, we were able to trace only very sporadically, they occured in the suprarenal gland, the kidney and the pancreas. -

According to the observations, respectively experiences made earlier, an increase in liver cysts, respectively the cyst-liver in the treated groups could have been understood as an expression of the proliferation-triggering effect of smoke-substances in the area of the liver, this was not recognizable (chart 46).

As in a second essential change, we were interested in the appearance of parasitic granulomatoses, respectively other inflammable liver diseases and bile-duct inflammations respectively bile-duct proliferation. These changes could yield information on toxic damages of the liver parenchyma, respectively on the triggering of proliferative changes which, with the effect of weak carcinogens, often precede the development of cirrhosis and carcinoma formation. Chart 47 shows the frequency distribution of these diseases. Neither ~~Neither~~ the frequency of hepatitis (which also contains granulomatous forms)

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

nor that of the liver abscesses or that of cholangitis shows a correlation to the treatment. Liver necroses which did not occur at all frequently, were very rarely observed in the smoke-exposed animals.

In the frequency of the hemosiderin deposits of the liver (particularly in Kupffer's Starcells), it is conspicuous that the animal groups I2 - I8A, included into the test later, respectively delivered later, are almost free of hemosiderin deposits. There are no significant differences between the smoke-exposed groups 4, 5 and 6 and contro group 3 ($X^2 \leq 2.02$ with 3 FG; (P> 5 %)). There is also no significant difference in the hemosiderin frequency between males and females ($X^2 \leq 0.30$; I FG; (P> 5 %)). Bile-duct proliferations which are often an expression of the first reaction of the liver tissue did not occur more frequently in the treated animals. A liver cirrhosis was not observed in any animal.

Liver tumors occured only with minimal frequency in our stock, chart 48.

It is a case of typically metastasizing bile-duct carcinomas (ill. 26), malignant hepatomas (ill. 27), of hemangioendotheliomas and of benign tumors like hemangiomas and papillary bile-duct adenomas. The total number of the occuring benign tumors does not allow for an indication of a change caused by the treatment. Solely in malignant liver tumors, a frequency increase ensues in the male animals treated with DMBA.

The frequency of liver cirrhoses, respectively primary liver tumors or bile-duct tumor was obviously much higher in other hamster colonies. CHESTERMAN and POMERANCE described 1965 a frequency increase of liver cirrhosis up to 20 % whereby 6 % of the animals with cirrhoses of the liver had liver tumors. FORTNER (I957 a, I958) among others, reported on spontaneous tumors among 620 hamsters

= 20 liver and bile-duct carcinomas

FORTNER (I96I) among I8I hamsters

= 8 liver carcinomas

KIRMAN (I962) among 7200 hamsters

= 94 hepatomas
7 malignant hepatomas
4 cholangiomas
2 malignant cholangiomas

SICHUK, FORTNER, DER, RUSSFIELD (I966) among 79 hamsters

= I bile-duct carcinoma

TOMATIS, DELIA PORTA, SHUBIK (I96[illegible]) among 63 hamsters

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Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

= I angioma of the liver

LEE, TOTH and SHUBIK (I963) among 54 hamsters

≈ I cholangioma

TOTH (I967) among 200 hamsters

= 2 cholangiomas
2 cholangiocarcinomas

HORN and SIEMERT (I968) among I630 hamsters

= I carcinoma

KIRKMAN (I950) among 5I hamsters

= I hepatoma

DELLA PORTA, SHUBIK and SCORTECCI (I959) among I5I hamsters

= I cholangioma

FORTNER and GALE (I958)

= 2 bile-duct adenocarcinomas.

After DMBA treatment, I cholangiocarcinoma among 24 hamsters was proven in the experiments of RAPPAPORT, PIETRA and SHUBIK (I96I). SHUBIK and Assoc. (I962) found after DMBA treatment of the skin (I02 animals), I cholangiocarcinoma and I cholangioma, after endotracheal application I cholangioma. They had also described tumors of the trachea, the larynx, the bronchi, the skin, the stomach, the intestines and the mamma as well as the ovaries after DMBA treatment. TOTH (I969) found among 60 hamsters, treated with DMBA over a longer period of time, I hemangiosarcoma of the liver besides 2 stomach sarcomas, 4 mamma carcinomas, 5 papillomas of the tubes and I hemangiosarcoma of the spleen.

We discussed the frequency of liver amyloidosis in the chapter "General Amyloidosis".

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Source: https://www.industrydocuments.ucsf.edu/docs/mkk0000

III.

2c Gastro-intestinal Tract

Because of the postulated association between the smoking of cigarettes and the formation of the peptic stomach ulcer (see also SCHIEVELBEIN, I968), we were particularly interested in the frequency of stomach ulcers in the different test groups of our test stock.

FORTNER reported I957 already on the spontaneous occuring of stomach ulcers in goldhamsters.

It is understandable that we were also interested in the question of the spontaneous frequency of benign and malignant stomach tumors in view of the varied treatment of the test animals.

There had been several reports on spontaneous stomach tumors in goldhamsters. FORTNER (I957, I958) described among 620 hamsters a total of I24 tumors (carcinomas, sarcomas polypi) in the gastro-intestinal tract, and I96I among I8I hamsters I2 omasum papillomas and 3 carcinomas (stomach). TOMATIS and Assoc. (I96I) saw 3 papillomas among I03 hamsters, DUNHAM and HERROLD (I962) I carcinoma among 360 hamsters, LEE and Assoc. (I963) 2 omasum papillomas among 54 hamsters, and TOTH (I967) 8 omasum papillomas among 200 hamsters, whereas KIRKMAN (I962) found among 7200 hamsters no stomach carcinoma but one duodenal polypus. Polypi of the glands of the stomach were described rarely (FORTNER I96I and DUNHAM and HERROLD I962). Polypi in the intestines were described by FORTNER (I96I), DELLA PORTA (I962) and SICHUK and Assoc. (I966). FORTNER and GALE (I958)found one adenocarcinoma in the intestines.

Stomach tumors were frequently observed after treatment with carcinogens. After oral application of DMBA, CHU and MALMGREN (I965) saw carcinomas of the omasum. After intravenous dispensal of DMBA, TOTH (I969) observed 47 papillomas of the omasum and I3 omasum carcinomas as well as sporadic polypi and carcinomas of the intestines among 60 hamsters. DELLA PORTA and Assoc, (I958) found after multiple intratracheal application of DMBA in one of 7 surviving hamsters an esophagus carcinoma.

We were able to prove the following changes in the individual sections of the gastrointestinal tract:

I. In the esophagus we found papillary growths of the pavement epithelium (ill. 28) only in the animals pretreated with DMBA, chart 50. Only once, group 8, we observed in a not-treated animal a beginning pavement epithelium carcinoma (ill. 29) of the mucous membrane of the esophagus. A correlation between treatment and tumor formation

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

in the esophagus could not be proven.

2. In the stomach we found with differing frequency fresh and older ulcerations of the mucous membrane which were already macroscopically recognizable in some instances. Chart 50 shows the frequency of the stomach ulcera in the individual test groups.

A significant increase of the stomach ulcera in the smoke-exposed animals, particularly in dependence from the dose, was not provable in comparison to the not smoke-exposed groups 3 and 8 ($X^2 = 8.49$ with 4 FG ($P > 5\ \%$)).

The frequency of gastritis too, did not differ between smoke-exposed and not smoke-exposed animals (chart 5I). Here, we only termed diffuse, strongly inflammable infiltrations of the mucous membrane of the stomach as gastritis.

In test and control animals, we observed typical stomach papillomas (ill. 30) of morbidly growing multi-layered pavement epithelium of the mucous membrane of the omasum. These stomach papillomas were unequivocally more frequently provable in the animals pretreated with DMBA. (Difference between the groups I, 2 and 3 : $X^2 = 203.4$ with 2 FG ($P < I\ \%$)). Chart 5 (next number illegible, Tr.) shows the frequency distribution of the papillomas of the stomach. Smoke-exposed animals showed no increase in stomach papillomas (difference between the groups 3, 4, 5 and 6 : $X^2 = I.36$ with 3 FG ($P > 5\ \%$)). Besides benign papillomas, we also observed infiltratively growing, to some extent, keratinized pavement epithelium carcinomas of the omasum (ill. 3I), (chart 50). Here too a greater frequency was evident solely in the male animals pretreated with DMBA (difference between the groups K, I, 2 and 3 : $X^2 = I0.4$ with 3 FG (P approximately I %). Carcinomas of the stomach glands were not provable. An increase of stomach tumors in the smoke-exposed animals, in dependence from the dose, was not provable in comparison to the control animals.

3. Intestinal Diseases: Among the intestinal diseases, unspecific inflammabel diseases

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

and invaginations must be separated from parasitic diseases and the intestinal disease which is termed "wet tail".

Upon dissection, a moderate to intermediate infestation with syphacia obvelata (oxyuridea) was found in several animals. The sexually mature worms were discovered in the coecum and colon.

A larger number of hamsters fell ill, approximately 15 to 20 days after their arrival with an extreme enteritis which presented itself as an ileitis terminalis, respectively a typhilitis and is known in the American literature as "wet tail" of the hamsters. Clinic and pathology of the disease are in accord with corresponding descriptions by SHEFFIELD and BEVERIDGE (1962), HANDLER (1965) as well as JONAS and Assoc. (1965). Frequency, see chart.

The disease starts with a diarrhoea which moistens the tail region of the animals. Within a few days the animals become emaciated and die within from 48 hours to one week after begin of the symptoms. Inflammations of the ileum and coecum are pathologically-anatomically prominent. They are combined with an extreme thickening of the intestinal walls, diptheroid coatings and ulcerations which perforate occassionally. Few animals survive the acute illness and die subsequently of complications like adhesive peritonitis, abscesses in the mesentery, scar-strictures as a result of the ulceration.

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Histologically recognizable is first of all, in the area of the mucous membranes of the ileum and coecum, an intermediate to extreme inflammable infiltration of the mucosa with desquamation of the epithelium and formation of diptheroid coatings. This is followed by a regenerative, strongly hyperplastic new formation of epithelium with distinct cell polymorphy and numerous mitoses (ill. 32 a - c). This pattern caused JONAS and Assoc. (1965) to speak of an enzootic adenocarcinoma. Numerous necroses of the mucosa were found besides the hyperplastic changes, they can focally extend through the muscularis as far as the serosa.

Source: https://www.industrydocuments.ucsf.edu/docs/nbkc0226

No specific viruses could be indentified through the bacteriological examination. Neither prohylactically, as SHEFFIELD and BEVERIDGE (1962) suggested, nor therapeutically was it possible to exert an influence on the course of the disease with neomycin-sulfate. In the initial phase of the test, a total of 489 animals per group died of "wet tail".

A relatively frequent cause of death were intestinal invaginations. Occassionally, they were combined with inflammable changes, but in most instances, they occured in already emaciated animals of advanced age.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlkk0000

III.

2d Cardiovascular System

Numerous researchers have been concerned with the question of the effect of smoke-substances on the heart and the circulatory system.

While, up to a few years ago, nicotine was predominantly held responsible for the supposable effect of smoke-substances on man (see AUERBACH and Assoc. I965), CO is being included into the discussion as an essential factor, to an increasing extent, through the experiments of ASTRUP and Assoc. (I970) as well as other researchers, eventhough the findings postulated for the rabbit did not occur in other animal breeds (monkeys, dogs, rats and mice) with an, to some extent, extreme CO-concentration (THEODORE and Assoc. I97I).

Through chronic application of nicotine, particularly in combination with an atherogenic diet, an increase of the tendency to arteriosclerosis was observed in the rabbit. The test results after experiments with pure nicotine application are very varied. SCHIEVELBEIN and EBERHARDT (I972) drew the conclusion, "that nicotine alone, even in a high dose, cannot produce any degenerative vascular changes, even in a species very susceptible to developing an arteriosclerosis, but that it shows an effect as an additional factor" (lit. in: The Health Consequences of Smoking I97I/I972). After intoxication with CO, an accumulation of arterioscleroses was observed in the rabbit (ASTRUP and Assoc. I967). Literature in : The Health Consequences of Smoking I97I, I972, SCHIEVELBEIN and Assoc. (I970). After chronic smoke-treatment of dogs, AUERBACH and Assoc. (I97I) saw an increase of sclerotic vascular changes particularly on the heart. Upon critical evaluation of the entire test results with nicotine, CO and total smoke treatment, it becomes evident that the effect obviously fluctuates extraordinarily from one animal breed to another and is largely dependent on the food supplied to the animals.

Earlier observations on inflammable vascular diseases after nicotine influence (lit. see LARSON and Assoc. I96I) are difficult to evaluate because a comparison with contr[illegible] groups of the same age is often missing in the tests. This holds true, to some extent, for examinations of not inflammable vascular diseases, particularly in larger control groups of dogs (CHEVALIER and KERSTEN I970) where vascular wall changes (hyalin-fibro[illegible] swelling) were observed in statistically significant frequency with increasing age.

A number of experiments was concerned with the sebacic acid metabolism of the animals after nicotine treatment as well as total smoke treatment. While predominantly increa[illegible] of the free sebacic acids, but to some extent also an increase in the cholesterol level were observed in man, the experimental tests show very varied findings for the effect of nicotine and total smoke (lit. in: The Health Consequences of Smoking, I97I, I972 as well as KERSHBAUM I967).

Already earlier, we reported (REICKZEH and DONTENWILL I970) on an increase of the erythrocytes after chronic smoke-treatment of the goldhamster. We attributed this erythrocyte increase to the chronic co-intoxication which takes place in our smoke-treatment method through the interval-free smoke inhalation lasting over a longer period of ti[illegible]. Our experiments DONTENWILL and Assoc. (I967) demonstrated, that with the high-dose smoke-treatment, up to 20 % CO-Hb is produced daily, which means, quantities which are not observed in man even in extreme instances. The persistent relative oxygen deficit is obviously compensated in the hamster through an increase of the erythrocytes.

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Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

That proves that a maximal CO-inhalation took place in the technique which we chose, and that we can also count on a high inhalation of other smoke-substances.

Since, as we were able to observe already earlier, a severe vascular amyloidosis of the kidneys with, to some extent, considerable shrinking appears in the hamster with increasing age, which must necessarily lead to a resistance rush, several components like nicotine, and CO-effect as well as hypertonia concur in the test arrangement. This was the reason why it seemed informative to examine the effect of the smoke-treatment on the sebacic acid metabolism in order to be able to better evaluate the postulated findings (see chapter 3).

Vascular wall changes in the sense of an arteriosclerosis were observed in the hamster after a "cholestrol diet" by ALTSCHUL (1946, 1950) and DEUEL (1947). Other authors like GOLDMAN (1950) were not able to confirm these experiments. Whether the observations of ALTSCHUL (1946) are cases of an arteriosclerosis, is doubtful, since a part of the vascular changes corresponds fully with the vascular amyloidoses observed by us. HANDFORTH (1962) described necrotizing arteritis and heart infarcts in the hamster after treatment with isoproterenol. FORTNER (1957) observed perivascular inflammations, hyaline vascular necroses of the arterioles, vascular thromboses as well as inflammable diseases of the myocardium and endocardium as spontaneous diseases in the hamster.

In our tests, we predominantly examined four vascular sections: vessels of the heart and the lung, changes on the aorta ascendens as well as stomach aorta and the pelvic arteries.

The vascular changes observed in all four sections were essentially cases of <u>inflammable vascular diseases</u>. These showed a vastly uniform pattern. They could be summarized under the term of thromarteriitis. All the morphological changes, also observed in man, which were described under these disease designations, like fibrinoid swelling, fibrinoid necrosis, proliferation of the endothelium, inflammable infiltration in the vascular wall and vascular vicinity were provable in the same way as beginning aneurysmatic bulging of the vascular walls. This is easily recognizable by comparisons on hand of the evaluations (charts 53 and 54). Inflammable vascular changes were in the lung more frequently combined with vascular thrombi, see chapter Respiratory Tract. 1002968612

<u>Chief localization of the inflammable vascular diseases were the large coronaries</u> (ill.33)

Source: https://www.industrydocuments.ucsf.edu/docs/xxxx0000

and the pelvic vessels (ill. 34). Inflammable changes of the aorta ascendens were observed only very rarely, but they were often severe and, predominantly in the chest section, combined with aneurysms respectively ruptures of the aorta which is demonstrated by illustration 35. The relatively small frequency of inflammable vascular diseases complicates the evaluation of the question, whether the smoke-treatment has led to an increase in vascular diseases. The inflammable diseases of the coronary vessels were in the female animals highly significant , with variances more frequent than in the male animals ($x^2 = 54.9$ with I FG; ($P < I\ \%$)). In female animals which show a shorter survival period - apparently due to frequent inflammable diseases - the number of coronary inflammations is, to some extent, distinctly higher in the smoke-exposed animals as compared to the not-treated animals. This holds true particularly for the groups I4, I5, I6 and I8 in comparison to control group 3. However, the frequency in group 6 is not significantly higher in contrast to group 3 ($x^2 = 2.I5$ with I FG; ($P > 5\ \%$)). The number of coronary inflammations after smoke-treatment is not higher with certainty in the male animals. The frequency rests in almost all the groups approximately at I %. Solely in group I5, 5% of the male animals had coronary inflammations. Inflammations of the large vessels of the pelvic and the upper thigh also occured unequivocally more frequently in female animals ($x^2 = 37.7$; I FG; ($P < I\ \%$)). An unequivocal frequency increase in the smoke-exposed animals as compared to the controls (groups K, 3 and 8) was not provable ($x^2 = I4.73$ with 5 FG; ($P > I\ \%$)).

In contrast to that, we were able to prove calcifications of the vessels of the media only in one animal of group K in the area of the aorta (ill. 36) and the large vessels of the pelvis. In examining the mentioned vascular sections, we saw no clues which would point to unequivocal vascular changes, comparable to arteriosclerosis, as they are described, analogus to man, in the monkey or the pig.

Among the observed diseases of the heart, two groups must be distinguished:

I. inflammable heart diseases,

2. degenerative diseases of the heart muscle.

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Source: https://www.industrydocuments.ucsf.edu/docs/...

Among the inflammable heart diseases, diseases of the cardiac valves stand in the foreground. We compiled their frequency distribution in the subsequent chart 55. These cardiac valve diseases are cases of endocarditis in all degrees (ill. 37 a, b and c) beginning with a swelling of the cardiac valves and inflammable infiltration to a maximal polypoidi coating of thrombi which, in parts, completely block the flow-out path. Chronic endocardites with fibrosis and calcification were also observed. Chief localization of the endocarditis were, in the same sequence as in man, the mitral valve and the aortic valve and the tricuspid valve. Occasionally, the inflammable valve changes also extended to the mural endocardium, from the aorta valve also to the aorta. In a large number of animals of all the groups, extensive thrombi were found predominantly in the left atrium (Vorkammer) (Ill. 38) which often filled the vena cava inferior (Vorhof) and the auricle (Herzohr) completely. An obvious correlation between these thrombi and the valve thrombi is provable only in one of the cases. There is no correlation to the treatment.

Besides these inflammable valve changes, we observed the already described changes in the sense of the arteriitis and periarteriitis of the coronaries. Thereby we counted fibrinoid swellings among the inflammable vascular ~~changes~~ diseases since they must be considered preliminary stages of these inflammation processes according to general experience.

Inflammable vascular changes of the aorta were rare and occured only in female animals. This fact demonstrates a parallelity to the vascular inflammations of the heart which occured more frequently in female animals. Aneurysms of the aorta were also more frequent in female animals. Parietal thrombi of the aorta occured only in one control animal. Besides the described inflammable vascular changes, vascular processes within the scope of an amyloidosis were prominent, as we described them particularly in the liver, kidney, spleen, suprarenal gland and in the testicles (see page).

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In this context we were interested in the comparison between body weight, heart weight, kidney weight. The comparison between body weight and heart weight (charts 56 - 63) as

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

well as kidney weight was intended to give a clue on the presence of a rise in blood-pressure. An increase of the heart weight with hereditary muscle degeneration was described by ONISHI and Assoc. (1970). The mentioned correlations are discussed in the following.

Correlation between Heart Diseases, End-Weight, Survival Period, Heart Weight, Kidney Weight

In the charts 56 - 63 the average end-weights, survival period, heart weights and kidney weights are listed for the animals in which the corresponding pathological finding was diagnosed, they are listed for 4 heart diseases (inflammation of the mitralis, inflammation of the aorta valve, inflammation of the myocardium, necrosis of the myocardium). The average values are calculated separately for the different test groups and the sexes. Only such groups were included in which at least 2 animals demonstrated the stated pathological finding. As a comparison, the weight, respectively the survival period for the total number of animals in the group in question, was listed in each instance.

In the inflammation of the mitralis which occured most frequently in the individual group there appeared no unequivocal difference between the average weight in the partial population of diseased animals and the total average weight. The average values of the partial population fluctuate irregularly around the corresponding total average, whereby deviations upwards are more frequent than deviations downwards. The differences however, are not significant which is attributable in some parts to the low number of animals in the partial test samples.

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Somewhat more unequivocal is the finding for the average survival period which rests above the total average in all groups for the animals with mitralis inflammation. The differences are in parts considerable and are significant particularly in the females (e. g. in the group K: 4, 6, 8, 16). The animals with inflammation of the mitralis have a longer survival period than the rest of the animals.

Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

In the heart weight, the average value for the animals with mitralis inflammation rested in almost all the groups above the total average. Only the groups 6 and I3 were an exception. The differences were however mostly not significant. Particularly high differences were found in the groups I, 5, 7, I2, I6, I8 and I8 A.

In the kidney weight, the differences between the partial population with mitralis inflammation and the entire population were less distinct. The values in the population with mitralis inflammation however, rested here too mostly above the total population values. An influence of the treatment could not be established. As far as weight differences were present, they should be predominantly attributable to the more advanced age in the group with mitralis inflammation.

Inflammations of the aorta valve were missing in the groups 2, 4, I3, I6, I7 and I8 A. In the remaining groups, frequently only 3 or 4 animals showed inflammations of the aorta valve. Only among the male animals of group I, there were 6 cases. The comparison of the average values was naturally very uncertain with these low numbers of animals. Therefore, differences could not be ascertained statistically, It is however conspicuous, that the end-weight in the pathological group rests mostly under the total average. The average survival period however, is mostly somewhat higher than in the entire group but the differences are not significant. It is conspicuous, that in group I, the heart weight of the female animals with aorta valve inflammation is considerably higher than the average heart weight of the female animals in this group. However,the kidney weights of these animals rest below the total average. This is an indication of a negative correlation between heart weight and kidney weight, however it could not be statistically ascertained (only two animals showed a pathological finding in this group).

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In the animals with inflammation of the myocardium, it is conspicuous that they have, particularly in the groups K, 2 and 8, a considerably higher end-weight than the total number of animals in these groups. The heart weight and the kidney weight in these groups also climbs above the average weight. However, the number of animals in the pathological

Source: https://www.industrydocuments.ucsf.edu/docs/nkbc0000

group is too small to obtain statistically significant results.

In the animals with necroses of the myocardium, the average survival period is significantly higher than the average value in the entire group. The heart weights are also in almost all groups higher for the pathological animals than for the rest. In the kidney weights, no unequivocal statement can be made on the differences to the total average.

Because of the examinations of the myocardium and the cardiac valves carried out on all animals, it was possible to exclude valve and myocardium diseases as factors for the enlargement of the heart.

In the pathological changes of the muscles of the heart, we made a basic distinction between dystrophic changes of the muscles of the heart which occur in parallelity to skeletal muscle dystrophy and are described in section III, 2 e, and between the myocarditis and severe purulent pericarditis (once among control, once in a treated animal)respectively heart infarcts. Myocardium inflammations were more frequent in the female animals and showed no correlation to the treatment. We observed larger heart infarcts in two animals (ill. 39). These occured in the controls with the same frequency as in the treated animals.

In the discussion of the pathology of the heart, blood-forming foci with bone formation and marrow formation occuring in the heart, should be mentioned. In the myocardium of the septum atriorum, there is normally a cartiladge disc in the area of the base of the aorta. It will ossify occasionally in older animals whereby blood-forming foci may occur in the area of ossification.

Besides inflammable and degenerative diseases of the cardiovascular system, tumors play a minor part. We found malignant hemangioendotheliomas (ill. 40) and benign hemangiomas (ill. 41)as they were described in similar form in the mouse and the rat, also in the area of the

spleen (see chart 64)

liver (see III/2 b)

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skin - soft tissues (see III/2 k)

uterus (see III/2 h).

Similar tumors had been sporadically observed in the hamster already earlier. FORTNER (I958) saw among 620 hamsters I3 hemangiomas of the spleen, DELLA PORTA (I962) among I9 hamsters I angioma of the spleen, KIRKMAN (I962) among 7200 hamsters 8 hemangiomas of the spleen, LEE and Assoc. (I963) among 54 hamsters 3 hemangiomas of the liver (2) and spleen, SICHUK and Associates (I966) among 79 hamsters I hemangioma of the spleen and TOTH (I967) among 200 hamsters 2 hemangiomas of the liver. In addition, LINDT (I958) described one angioma, VALENTOVA and Assoc. (I962) and KESTERSON and CARLTON (I970) each one hemangioendothelioma.

Upon chronic treatment with DMBA, hemangiomas (liver, spleen, lymph nodes, skin) and hemangioendotheliomas (kidney) were observed by TOTH (I969), RAPPAPORT and Assoc. (I96I) as well as LEE and Assoc. (I963). While malignant vascular tumors occured only sporadically upon treatment with DMBA, RABSON and Assoc. (I960) saw after intratracheal application of polyoma virus among 22 hamsters 9 hemangiomas ~~xxxxxxx~~ of the liver besides 6 pavement epithelium carcinomas of the lung.

We found in our experiments no correlation between the formation of the tumors of the cardiovascular system and the treatment. We assume that all discovered tumors are cases of spontaneous tumors.

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

III.
2 e Dystrophic Myopathy - Cardiomyopathy

Spontaneously appearing degenerative changes in the skeletal muscular system of the goldhamster were apparently first observed by ALTSCHUL (1946). There were already earlier reports (HOUCHIN 1942) on the frequent appearance of muscle degeneration in the goldhamster due to vitamin E deficiency. HOMBURGER and Assoc. described 1962 a primary degenerative disease of the myocardium and skeletal muscle in an in-bred stoc of Syrian goldhamsters. HOMBURGER and BAJUSZ (1970) reported later on a varying occurance of muscular dystrophy in different hamster breeds. The degenerative muscular diseases which often occur simultaneously in the skeletal muscular system and the muscular system of the heart, show considerably varied patterns. Fine granulous calcareous deposits are in parts recognizable in the cloddy, crumbled muscle fibers, in parts, v have simultaneously cloddy decay of the muscular system, so-called Zenker Degeneration, respectively muscle fiber necroses and extensive regenerative processes with connective tissue and cell proliferation as they are also described in human muscular dystrophy and different myopathies of domestic animals (ill. 42 a - c). In the degenerative changes of the myocardium, one often recognizes side by side, all stages of fresh muscular necroses and ossification to connective tissue morbid growth and scar formation. The electronmicroscopic picture of the changes of the muscular system was described by ONISHI and Assoc. (1970)

According to the research of HOMBURGER and BAJUSZ (1970), the changes of the skeletal muscular system as well as those of the myocardium are cases of a genetically conditioned illness which is bound to the X-chromosome. Chart 65 shows the frequency of the degenerative muscle diseases in the skeletal respectively the cardiac muscular system. An influence of the treatment on this hereditary illness could not be proven.

It is conspicuous, that the frequency of cardiomyopathies is considerably larger in the groups I2 - I8 A than in the groups K - II. However, the frequencies for cardiomyopathy as well as for dystrophic myopathy within the groups I - II and within the

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

groups I2 - I8 do not differ significantly from each other. Since all animals (K - I&A) came from the same breeder and the same colony, only the time of the test begin could be discussed as a reason for the differing frequencies. The groups K - II were delivered in spring (February to March) and the groups I2 - I8 in fall (September). The tests were begun accordingly.

An influence of the smoke-treatment is not provable.

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III.
2 f Kidney and conveying Urinary Organs

In the postulated correlation between inhalation of cigarette smoke-components and tumor formation in the bladder - literature see SCHIEVELBEIN and ZICKGRAF (1968) - a more exact examination of the mucous membrane of the bladder seemed necessary in the test animals. Since the animals had absorbed a high concentration of smoke-components, a tumor-triggering effect in the bladder was within the range of possibilities.

The evaluation of the findings of the histological examination of the bladder showed, besides sporadically occuring inflammable diseases of the bladder wall and the mucous membrane (a total of three cases of severe bladder inflammations in groups 6, 8 and I), only in one animal of group I8 A (nitrosamin without smoke-treatment) a typical, mildly papillary carcinoma of the bladder (ill. 43) with beginning wall infiltration.

Spontaneous bladder carcinomas have so far not been described in the hamster. Through treatment with o-aminoazotoluene, it was possible to produce bladder carcinomas in a frequency resting far higher than in other test animals (TOMATIS and Assoc. 1961).

Tumors of the kidney were also extremely rare in our stock. We found in one treated animal (group I8 - smoke-treatment - died before the additional treatment with nitrosamin) one renal pelvis carcinoma (ill. 44) with, in parts, papillary structure of the distinctly infiltratively growing transitional epithelium.

Spontaneous kidney tumors had been sporadically observed already earlier by FORTNER and GALE (1958), FORTNER (1957, 1958), DUNHAM and HARROLD (1962), FORTNER (1961), TOMAN (1963), BODIAN and RIGBY (1964), TOTH (1967). They were cases of adenocarcinoma and of nephroblastomas (Wilm's Tumor). That different carcinogens are tumor-producing in the kidney of the goldhamster (nitrosamins - TOMATIS and Assoc. 1964; benzpyrene - DONTENWILL and RANZ (1960) was proven by the mentioned authors.

Besides the described tumors and the diseases of the kidney, discussed under amyloidosis, a small number of inflammable kidney diseases (nephritis, pyelonephritis) were found independent of the test arrangement (chart 66). We should also mention the sporadic occurances of kidney cysts (2 cases in group K, one case each in group I and

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

group 10), (see cysts of the liver).

A comparison between kidney weight and heart weight seemed significant to us, particularly in view of the appearance of "arteriosklerotic" or inflammable vascular diseases, respectively the formation of renal high pressure. STROLA and Assoc. (1954) had proven that a renal ischemia in the hamster leads to a rise in blood-pressure.

In the charts 67 and 68 are compiled the average heart and kidney weights of the different test groups for the stages 1, 2 and 3 of kidney amyloidosis, see chapter "Generalised Amyloidosis". It is conspicuous that the average kidney weight of the animals with an amyloidosis-stage 2 is higher in all groups than the corresponding weight for the total number of the animals; the kidney weight of the animals with amyloidosis-stage 3 however, is in most groups lower. It was possible to ascertain the differences between stage 2 and stage 3 with overwhelming statistical evidence (with a probability of error of 5 %). In the females of group 5 as well as in the males of the groups 3 and 6 , a difference in kidney weight between stage 2 and 3 could not be ascertained statistically.

The heart weights on the other hand did not show any distinct dependency on the amyloidosis-stage of the kidney. In most groups the average heart weight in stage 3 rests under that of stage 2. But the differences are mostly not significant. Only in group 6 ♂, the difference between stage 3 and stage 2 is statistically ascertained. In several groups (K ♂, 1 ♀, 2 ♀, 4 ♂ 5 ♀ and 9 ♂), the weight is higher in stage 3 than in stage 2, however this increase could not be ascertained.

For a further analysis of this correlation, we investigated the correlation between difference in body-weight and kidney-amyloidosis. In the charts 69 and 70 are listed the average weight differences (between end-weight and initial weight) for the animals with the different amyloidosis-stages. Almost all groups show here a distinct negative dependency of the weight difference and thus of the end-weight from the amyloidosis-

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stage; that is, the end-weight of the animals is considerably lower in stage 3 than in stage 2 or I. Particularly great (and highly significant) is the difference in the groups K, 3, 4 and 5. In the smoke-exposed group 6 on the other hand, the difference between stage 2 and stage 3 is only minor and statistically not significant. The influence of the kidney-amyloidosis on the weight of the animal is here obviously covered over by the influence of the smoke-treatment.

The dependency of the end-weight from the amyloidosis-stage suggests to calculate and examine not only the absolute heart weight but also the relative heart weight (relative to 100 g body-weight)for the three amyloidosis-stages. These relative heart weights for the different groups and stages are listed in the charts 71 and 72. It becomes evident from these charts that these relative heart weights in the groups K, I and 2 as well as 5 are distinctly higher in stage 3 than in stage 2 or stage I. In group 3, the relative heart weight is higher only in the males. In group 6 on the other hand, in males and females, the relative heart weight in stage 3 is distincly lower than in stage 2 and (only in the males) in stage I. In the remaining groups there is not distinct difference in the relative heart weight between the amyloidosis-stages.

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III.
2 g Generalized Amyloidosis

In earlier research on the amyloid formation in the goldhamster, (DONTENWILL and Assoc. I960) we had reported on the frequent appearance of amyloidosis (up to I00 %) particularly in the old age of the animals. These findings have meanwhile been confirmed by GLEISER and Assoc. (I97I) and BLAHA (I967) and others. We had termed this form of the amyloidosis as a primary amyloidosis and had compared the genesis of the amyloidosis disease to the primary amyloidosis in man.

While we did not undertake a division in stages in the evaluation of the amyloidosis of the spleen, the liver and the suprarenal gland, despite considerable differences in the degree of amyloid deposists, we did distinguish between three stages of severity (chart 73) in the kidney-amyloidosis, particularly in view of possible effects on the heart and the vascular system (see under Kidney and Cardiovascular System):

I. a moderate to intermediate amyloid deposit (ill. 45 a) in the area of the glomerulus loops, and an amyloidosis of Bowman's Capsule with only a minor nephrotic component,

2. a strong amyloidosis (ill. 45 b) of the glomeruli and the basal membranes of the tubuli as well as the interstitial vessels with considerable amyloid-nephrosis,

3. an extreme-severe amyloidosis (ill. 45 c) of the kidney with vast shrinking of the kidney parenchyma, a strong nephrotic component and increasing lack of functioning of the nephrons.

A predominance of the female animals became evident in stage 2, as it had been for the liver, spleen and suprarenal gland. The difference between males and females was statistically examined with an X^2- test, and the differences were very highly significant (X^2= I06.2 with I FG). An influence of the smoke-treatment was not recognizable, not even in the male animals with a longer survival period. The differences between the groups 3, 4, 5 and 6 as well as 8 were statistically only weakly ascertained with an

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X^2- test ($X^2 = 10.7$ with 4 FG, ($P < 5$ %)). However, the differences between these group were highly significant for the females ($X^2 = 32.5$, ($P < 1$ %)). A difference in frequency of the same extent was not provable between male and female animals in stage 3. ($X^2 = 3.56$, ($P > 5$ %)). A higher frequency was evident here only in individual smoke-exposed groups. In the females as well as in the males, the differences between the groups 3, 4, 5, 6 and 8 were highly significant ($P < 1$ %).

The amyloidosis of the kidney was frequently accompanied by strongly homogenous calcareous deposits in the tubuli clearances, respectively the collecting ducts.

The amyloid deposits of the liver (ill. 46) were as a rule most strongly provable in the periportal fields, as described already earlier. The greater frequency in female animals, despite a lower average age, is conspicuous in the amyloidosis of the liver as well as of the spleen (chart 74). The differences between males and females were in this case very highly significant ($X^2 = 114.7$ with 1 FG). On the other hand, the differences between the smoke-exposed groups 4, 5 and 6 and control group 3, respectively placebo group 8 were highly significant only for the females while they could not be ascertained for the males.

The amyloidosis of the spleen was predominantly a pulpa-amyloidosis. Similar to the liver, the differences between the two sexes were also highly significant in the amyloidosis frequency of the spleen. The differences between the smoke-exposed groups and the control group, respectively the placebo group were only in the females highly significant ($X^2 = 37.0$ with 4 FG) while they could not be ascertained for the males ($X^2 = 6.81$).

The amyloidosis of the suprarenal gland (ill. 47) often led to an extreme atrophy, as already described earlier. Here too, a greater frequency was evident in the female animals.

In the evaluation of the frequency distribution of the amyloidosis, we gave special

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consideration to the increase of the degree of severity with rising age in relation to the treatment of the test animals and to traceable inflammable changes of the organs and the tissue. We obtained neither unequivocal correlations to inflammable changes nor an unequivocal influence of the treatment on the frequency and degree of severity of the amyloidosis. The obtained findings once more confirm the opinion voiced already earlier, that the amyloidosis is a case of a hereditary spontaneous disease of the hamster which attacks over 90 % of the animals with increasing age.

In the charts 75 and 76 are listed for the different test groups and separated by sex the number and the percentage rate of animals which in the pertaining age groups (0 - 25, 26 - 50, 5I - 75, 76 - I00, $\geq$ I00 weeks) showed the stages I, 2 or 3 of a kidney amyloidosis.

The charts show (75 and 76) that the frequency and the degree of severity of the kidney amyloidosis depend unequivocally on the age. This becomes particularly evident in the frequency of stage 2 in the females which mostly rises from 0 % in the age group 0 - 25 weeks to 80 % up to I00 % in the age groups over 75 weeks. Moreover, the stages 2 and 3 are found predominantly in the higher age groups while stage I often appears only in the age groups under 75 weeks in the females. In the males on the other hand, stage I also occurs in the higher age groups (over 75 weeks) while it was practically not observed in the age groups from 0 - 25 weeks.

Altogether, the charts 75 and 76 show distinct differences between males and females which can not be explained through the different age distribution. Instead, the amyloidosis is generally less frequent in the males; it can be observed predominantly in the higher age groups and does indeed in its frequency show an age dependency but not in a degree of severity as does the kidney amyloidosis. All three severity stages occur in the age groups over 50 weeks with approximately the same frequency.

In the females however, we obtain a distinct dependency of the frequency and the degree

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of severity on the survival period.

An influence of the smoke-treatment on the amyloidosis frequency could not be ascertained, neither in the males nor in the females. Particularly the amyloidosis stage 2 is indeed the lowest in group 6 for males as well as females; but this effect may be attributable to the fact that in group 6 the average survival period was the shortest and that thus the animals did not reach an age sufficient for the formation of an amyloidosis.

We also found anoften severe vascular amyloidosis (ill. 48) in other organs like the pancreas, the thyroid gland and the testicles (see Gonads), respectively a para-amyloidosis which we did not evaluate quantitatively.

The stages of the amyloidosis are essentially correlated with the survival period and show no correlation to the smoke-treatment.

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III.
2 h Reproductive Organs

Different authors described a histopathologically realizable atrophy of the testicles, in dependence from the dose, after chronic nicotine supply, respectively effect of smoke substances in dogs, rabbits, guinea-pigs, rats, mice and young roosters. A division into stages of the histological testicle changes was thereby not possible since the obtained results demonstrated very great fluctuations. The age of the animals apparently played an essential part in the described testicle changes (literature by SCHIRREN (1963) as well as LARSON and Assoc. 1961). The existing reports do not give unequivocal findings on the extent of the influence on the gonads of smoke substances, particularly of nicotine, on account of the very varied animal stocks and the differing testing techniques.

GRANADOS and DAM (1948) described in one breed of hamsters (in-bred)testicle hypoplasias in varying age groups (92 - 240 days) and under varying feeding conditions. The testicle weight in the hypoplasias fluctuated between 0.002 and 0.306 g compared to a testicle weight of 0.900 to 1.400 g in the remaining animals. Among the "normal" hamsters, the researchers observed fertile animals with a testicle weight up to 0.580 g. KALOYANU-IORDEKEL and MIKLE (1966) found a clear decrease of the testicle weight upon reduction of the room-temperature.

In our tests, we purposely avoided a grade-division of the spermiogenesis disorders. We termed the changes atrophy, respectively hypoplasia if no spermatozoa or spermatids could be traced in the testicles. This proof is the simplest with Weigert's fibrin coloration in which the heads of the sperma turn a deep blue. This sharp delimitation seemed sensible to us, since an arbitrary gradation would have had to be selected in any grade-division, the evaluation of which would not have been possible with such an extensive stock of animals. We determined the weight of the testicles and undertook a histological examination.

The atrophied testicle shows in comparison to the normal testicle, as ill. 49 demonstrates, besides a reduction of the lumen of the testicle tubules, an extreme reduction of the individual layers of cells, whereby often only spermatogonias and primary spermatocytes remained visible. Parallel to that, the globus minor shows distinct signs of an atrophy and the seminal vesicles show a strong lumen reduction at the same time. In our experiments, we could not confirm the frequently,in the testicles of the rat, observed parallelity between a disorder of the spermiogenesis and the appearance of

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giant cells (COHRS and Assoc. 1958). Spermiocytes and giant spermid cells of the testicle tubules also occured in a largely intact spermiogenesis and were observed relatively rarely in extremely atrophic testicles, consequently they are not at all a regular finding in atrophy, to some extent, the atrophic testicles show patterns as they had been described by GIESE and HORSTEBROCK (1962) for quantitative lack of nourishment in man.

The evaluation of the findings of the testicles (chart 77) demonstrates that in all test groups, a, in parts, considerable atrophy becomes evident with increasing age, which is demonstrated by the reduction of the testicle weight as well as in the histological finding "atrophy". The critical value obviously rests at a testicle weight of circa 0.7 g. Almost all testicles below this weight mostly show an advanced atrophy. The average values of the relative testicle weights (relative to 100 g animal weight) have already been listed in the chart of relative organ weights. For a statistical evaluation of the testicle findings, we calculated for each group the average survival period and the average testicle weight as well as the maximal testicle weight for the animals with testicle atrophy. The results are compiled in charts 78 - 80. For the purpose of a comparison, we also listed the total average values for the survival period and the testicle weight of all animals of the groups in question in the chart.

It becomes evident, that the average survival period for the animals with testicle atrophy is in all groups considerably higher than that for all animals of the group in question. The difference amounts to up to 64 % of the total average (in group 16). A systematic influence of the smoke-treatment can not be established from the results of the chart. The average survival period is the longest in control group 3 for all animals as well as the animals with testicle atrophy. While however, in all animals of the smoke-exposed group 6, the survival period drops significantly, a similarly significant drop for the animals with testicle atrophy can not be traced in group 6.

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It is remarkable that the frequency of testicle atrophy in group 6 with 3I % is practically just as low as in group K with 32 %. Consequently, an influence of the smoke-treatment is also not traceable in the testicle atrophy. The chart shows furthermore that the average testicle weight in the animals with testicle atrophy rested considerably below the average testicle weight of all animals. It is particularly low in group 6, eventhough the total average is relatively high in this group. However, even in group 8, differences in the average testicle weight between the atrophy-animals and the remaining animals are still relatively great. Therefore, these differences cannot be attributed to the smoke-treatment. It is furthermore remarkable that, in control group 3, the testicle weight of the atrophy-animals is the highest with 0.4 g.

In chart 80 is compiled the frequency of testicle atrophy for the 5 different age groups (0-25, 26-50, 5I-75, 76-I00, >I00 weeks). For each age group and each group are listed the number n of the animals of this age group, the number n of the animals with testicle atrophy in the age group in question, and the percentage rate of animals with testicle atrophy in the age group in question. It becomes evident that this percentage rate definitely rises in each group with increasing age. While in the age group from 0-25 weeks not one single animal shows a testicle atrophy, more than half of the animals have testicle atrophies in the two highest age groups in almost all the test groups. The frequencies in the highest age groups (> I00 weeks) demonstrate greater fluctuations because of the small numbers of animals in the group. The age dependency of the testicle atrophy does obviously not depend on the treatment. However, the frequency of testicle atrophy varies in the different groups. An influence of the smoke-treatment is not recognizable.

In order to be able to determine more precisely the influence of the smoke-treatment on the relative testicle weight, we established the absolute and relative testicle weights in different test groups which were killed after a test length of I3 weeks. As chart 8I shows, it is thereby unequivocally evident, that through the remaining of the animals in the smoke tubes of the smoking apparatus without smoke-supply,

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

that is, with the same length of remaining in the smoke tubes and a supply of fresh air, the testicle weight is considerably influenced. While the average testicle weight of the control animals (with same age, same test length and keeping)without any treatment amounted to 3.23 g per I00 g body-weight, the animals treated in the apparatus with fresh air showed a strong reduction of the testicle weight. The reduction from 3.23 g to 0.72 g per I00 g body-weight is the equivalent of a reduction of 3/4 in comparison to the control animals. According to the experiences of other researchers, the testicle of the hamster is obviously very sensitive to outside influences. Whether the reduction of the testicle weight is caused by a shifting of the testicles into the abdominal cavity during the exposure to fresh air could not be determined. When one compares the test animals treated with fresh air to the smoke-exposed test animals, one finds in the smoke-exposed animals a reduction of the average testicle weight from 0.72 g to 0.47 g per I00 g body-weight. This difference is significant ($p \leq 0.00I$). However, the reduction is vastly smaller than in the comparison between the untreated and fresh-air controls (3.4 g to 0.72 g per I00 g body-weight values of the inidividual groups - see chart 8I). The present test results demonstrate that the hamster is obviously not a suitable test object on which to measure, in the present test arrangement, the influence of smoke-substances on the testicle weight, respectively the testicle function. The in itself already strong fluctuation of the testicle weight in the hamster complicates a comparative observation even without treatment, particularly in older animals. In the critical assessment of the total findings, an unequivocal effect of the smoke-substances on the testicle function is not ascertainable.

An extraordinary thickening of central vessels of the testicles was in some parts found in individual animals. Theses thickened vessels show a positive amyloid-reaction, in parts, the lumen of the vessels is hardly recognizable at all. Similar changes were described by GLEISER and Assoc. (I97I). The frequency of the vascular amyloidosis fluctuates very strongly, it is the highest in the animals treated only with asbestos

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or gas-phase. An influence of the smoke-treatment is not ascertainable.

Besides the described changes, there were individual cases of inflammable processes in the testicles and ossifications in the area of old inflammation foci.

Testicle tumors were not provable. Prostata tumors were also not observed.

Only sporadic spontaneous tumors in the genitals of the male hamster have so far been described by other authors. FORTNER (I96I) described among 94 hamsters 2 adenocarcinomas of the prostata, KIRKLAN (I962) I seminoma as well as 9 (testicle and globus minor) adenomas among 7200 hamsters, FORTNER and Assoc. (I963) I adenocarcinoma of the prostata.

In the ovaries , we found sporadically variedly-sized smooth-walled cysts, lined with regular epithelium (chart 77). In group I (DMBA and smoke) 20 % of the animals had cysts in the ovaries, in group 2 (DMBA) even 30 %. In control group 3 on the other hand, only 6 % of the animals had cysts in the ovaries. The difference between the groups treated with DMBA and the control animals is highly significant ($X^2 =$ I3.I2 with 2 FG, ($P <$ I %)). It is probable that DMBA damages the follicles which then degenerate into cysts,as KRARUP (I969) was able to demonstrate in mice. The smoke-exposed animals do not show a varying development of the follicle in the ovaries in comparison to the controls.

In the area of the female genitals, inflammable changes could be proven only in individual animals which, however, did not show a correlation to the nature of the treatment.

The observed tumors did also not demonstrate an increased frequency in the treated test groups as compared to the controls.

Among tumors must be mentioned benign papillomas of the mucous membrane of the corpus and the cervix (ill. 50) as well as hemangiomas of the mucous membrane of the corpus (chart 82).

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FORTNER (I96I) also observed similar tumors. TOTH (I967) found I adenocarcinoma among I00 hamsters.

Sporadically, we found small, easily delimitable leiomyomas of the corpus uteri (ill. 5) whose structure is vastly similar to the tumors described in man.

Individual cases of similar tumors were described already earlier by FORTNER (I957, I96I), FITE (I958), FORTNER and GALE (I958). TOTH (I967) describes I leiomyosarcoma among I00 hamsters.

Among the carcinomas of the uterus (chart 82), we found two types. Exophytically growing papillary corpus carcinomas and strongly infiltratively growing, more endophytically growing, adenocarcinomas of the mucous membrane of the corpus uteri (ill. 52).

Similar tumors had been proven already earlier by LINDT (I958), FORTNER and GALE (I9[illegible]) FORTNER (I958) (9 among 620 hamsters), FORTNER (I96I) (5 among 87 hamsters), KIRKMAN (I962) (3 among 7200 hamsters) and DUNHAM and HERROLD (I962) (3 among 360 hamsters). Only SHERMAN and Assoc. (I963) had reported of an adenocarcinoma of the cervix.

Ovarian tumors are rare in the hamster. We found only 3 small luteomas (ill. 53) and and I granulosa cell tumor with characteristic structure.

They were also described in similar form by FORTNER (I957, I96I) and KIRKMAN (I962) (2 among 7200 hamsters). Malignant ovary tumors were not provable. Other authors have also described individual cases of granulosa cell tumors (2 among 360 hamsters), (DUNHAM and HERROLD, I962)

The frequency of the tumors was so small that a statistical test does not make sense. It is however remarkable, that of the 4 observed benign tumors of the ovaries, three appeared in the animals treated with DMBA. An increase in the tumors in the genitals of exposed animals was not provable.

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III.
2 i Brain

Since the evaluation of the changes in the respiratory tracts was uppermost in the exploration, we decided on a histological examination of entire vertical sections of the head. These allow, as ill. 54 shows, for a survey of the mucous membranes of the cavity of the nose as well as the oral cavity, respectively the palate. At the same time, these sections permit an evaluation of the brain areas present on the same level.

Changes in the brain which we designated as calcareous glands occured relatively frequently. It was a case of globular, layered calcium secretions which occured predominantly in the bulbus olfactoris and were generally found in the area around small vessels (ill. 55). These calcium concrements could be the results of an inflammable virus infection according to the opinion of Prof. STOCKDORPH to whom we presented these preparations. Similar changes have also been described in the human brain (ERBSLOH, 1944).

The chart shows the frequency of the calcareous glands in the brains of the individual test groups. Reliable differences in frequency are not provable.

A meningitis or an encephalitis occured only in individual animals proceeding from phlegmons in the head region.

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Source: https://www.industrydocuments.ucsf.edu/docs/mkk0000

III.
2 k Skin and Subcutaneous Tissue

To the most frequent changes in the area of the skin belonged inflammable processes, particularly phlegmons and abscesses (chart 84). Their frequency was influenced neith by the smoke-treatment nor by manipulations in the smoke-treatment apparatus. Occasionally, we observed eczemas and alopecia.

The spontaneous tumor frequency in the area of the skin was insignificant, see chapte III/2 m and chart 97. We observed:

I pavement epithelium carcinoma with strong keratizing and metastases,
I basalioma, (ill. 56)
nevi (in parts subcutaneous tissue of the eye region),
malignant, metastasizing melanomas, (ill. 57 and 58) hemangiomas,
I cystadenopapilloma, (ill. 59)
I neurinoma,
I clear-cell adenoma,
I skin papilloma

The skin carcinoma, the basalioma, the cavernous hemangioma and the neurinoma showed a histological structure comparable to that of a human tumor. The melanomas show a very strong pigmentation and mostly spindle-shaped regular cells and resemble, for the most parts, the so-called blue nevi in man. The malignant melanomas show two types of cells with more spindly cells and much pigment and more round cells with less pigment.

The cystadenopapilloma, which we found, resembles tumors of the same name described in the exocrine glands of man; its starting point was not precisely determinable.

Similar spontaneous skin tumors had been described already earlier by FORTNER (1957) among 620 hamsters 8 melanomas, FORTNER (1961) among 181 hamsters 5 melanomas, DUNHAM and HERROLD (1962) among 360 hamsters I pavement epithelium carcinoma, KIRKMAN (1962) among 7200 hamsters I neurofibroma, 21 blue nevi and 7 malignant melanomas and TOTH (1967) among 200 hamsters I melanocytoma. Melanomas and nevi were also described by

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

CRABB and KELSALL (1952), FORTNER and GALE (1958), GREENE (1958), SHUBIK, PIETRA and DELLA PORTA (1960), SCHRÖDER (1961), BOMIRSKI and Assoc. (1962) and RAPPAPORT and Assoc. (1963). LINDT (1958) reported of one basal cell carcinoma and one pavement epithelium carcinoma. They belong to the most frequent spontaneous tumors of the hamster.

After local and intravenous treatment with DMBA, different skin tumors (hemangiomas, melanomas, papillomas, carcinomas, sebaceous gland adenomas, adenocanthomas, cystadenomas, sarcomas) were described: KRIEGEL (1954), DELLA PORTA and Assoc. (1956), HORNING (1958), SHUBIK and Assoc. (1960), RAPPAPORT and Assoc. (1961), LEE and Assoc. (1963), HALMER (1966), TOTH (1969), RAITSCHEW (1970) and numerous others.

An increase in the tumors of the connective and supportive tissue, respectively the skin through the treatment was not provable (see also chapter: "Tumors of the Connective and Supportive Tissue").

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Source: https://www.industrydocuments.ucsf.edu/docs/mkk00036

III.
2 1 Tumors of the Suprarenal Gland

Among the relatively rare spontaneous tumors of the hamster, the relatively high frequency of suprarenal gland tumors had already been recognized early.

ASHBEL (I945) saw among I000 hamsters I0 suprarenal gland tumors, FORTNER (I957, I958) among 620 hamsters a total of I2I tumors(carcinomas, adenomas), FORTNER (I96I) among I8I hamsters 53 adenomas and I2 carcinomas, KIRKMAN (I962) among 7200 hamsters 550 adenomas and 3I adenocarcinomas, I ganglioneuroma (cortex), LEE and Assoc. (I963) among 54 hamsters 4 adenomas, SICHUK and Assoc. (I966) among 79 hamsters I7 adenomas, DUNHAM and HERROLD (I962) among 360 hamsters 6 carcinomas and frequently hyperplasias, TOTH (I967) among 200 hamsters 2 adenomas and 2 carcinomas, KIRKMAN (I950) among 5I hamsters 4 adenomas, FORTNER and GALE (I958) 2 carcinomas (number of animals not stated).

HOMBURGER and RUSSFIELD (I970) described in a new in-bred breed of Syrian Goldhamsters an occuring of suprarenal gland tumors provable up to 50 %. Thereby they distinguish essentially two types of kidney tumors, which they term A-B cell tumors and cortical tumors. The one form of tumors is described as more spindle-cellular while in the other roundly oval cells, that is, a tumor structure, respectively a cellular structure, respectively a structure of cells is being described which is more suggestive of the zona fasciculata of the suprarenal gland. The authors held genetic factors responsible for the formation of this high frequency of suprarenal gland tumors. The different tumor forms show, according to MURTHY and RUSSFIELD (I965 and I966) a very varied histochemical behavior and a varied hormonal effect.

We ourselves have distinguished between two forms of suprarenal gland tumors: spindle-cellular tumors (ill. 60) whose cell form and structure of cells is suggestive of the zona glomerulosa and

round-cellular (ill. 6I and 62) to oval cortex tumors which in their structure and their cell forms resemble more the zona fasciculata. The distinctly polymorphous adenoma form show mostly a round-cellular, respectively a fasciculata resembling structure and were counted among these. This division does not contain a functional equating of the tumor with the morphologically similar cortex tissue. We observed in individual animals both tumor forms at the same time in the cortex of the suprarenal gland, and have not, like HOMBURGER and RUSSFIELD (I970), listed hyperplasias and adenomas separately, since, upon examination of larger series, we arrived at the conclusion that the adenomas develop from small cortical nodes which HOMBURGER and RUSSFIELD (I970) had termed hyperplasias

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Source: https://www.industrydocuments.ucsf.edu/docs/snbx0000

We termed only those tumors malignant which demonstrated an unequivocally infiltrative growing process into the neighboring areas and showed metastases (ill.63 a and b). In the evaluation of the examinations, we were interested in the sexual distribution of the tumors, the age dependency and the dependence on the nature of the treatment. Large tumors reached weights to above 2 g. Smaller tumors were not distinguishable from the weight of the suprarenal gland. With larger tumors, we found in the not-affected suprarenal gland a, to some extent, unequivocally provable compensatory atrophy. This becomes very clearly evident in a compilation (chart 85) in which appeared, in parts, one-sided tumors, and, in parts, two-sided tumors, whereby individual tumor-free suprarenal glands demonstrated a reduction of their weight to approximately I/4 of the average weight. Benign suprarenal gland tumors also ~~often~~ showed an often considerable polymorphy of the nuclei. A correlation between amyloidosis and appearance of suprarenal gland tumors was not provable.

The frequency of suprarenal gland tumors (chart 86) was considerably lower in the female animals, a finding which was equally provable in the treated animals and the controls. We observed: benign tumors of the cortex (spindle-cellular) in females 3, in males 146; benign tumors of the cortex (round-cellular): in females 73, in males 340; malignant tumors of the cortex: in females I, in males 5. The differences are highly significant.

For the round-cellular tumors of the males, there are also highly significant differences between the groups 3, 4, 5 and 6 (X^2=23.I8; 3 FG) in the sense of a smaller frequency in the smoke-exposed animals. These differences are lacking for the spindle-cellular tumors. The round-cellular fasciculata resembling tumors of the suprarenal gland were more frequent than the spindle-cellular tumors of the suprarenal gland (4I3 : I49). A positive correlation resulted between the appearance of suprarenal gland tumors and the identification of a testicle atrophy. Whether this correlation is a case of a direct dependency or of a so-called pseudo-correlation, which is, in this

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

instance, attributable to the survival period with which both disease frequencies are highly correlated, remains to be seen.

An increase of the suprarenal gland tumors in the smoke-exposed animals was not provable.

Charts 87 - 96 show the age dependancy of the suprarenal gland tumors. We determined and listed in the chart for each group the number and the percentage rate of animals with tumors (spindle-cellular, round-cellular and malignant tumors of the cortex) in the 5 age groups (0-25, 26-50, 5I-75, 76-I00, > I00 weeks). A distinct age dependency becomes evident. While in the age group 0-25 weeks no tumors appeared at all, and in the age group 26-50 weeks tumors occured only very rarely, the tumor rate (in males) in the age group over I00 weeks often runs higher than 50 %.

Twice (groups I and I3, that is, not smoke-exposed animals) we found adenomatous growth of the marrow with cyst formation of the regular cells. The cysts were filled with secretion, the adenomas, which we termed cystadenomas, were very small. Similar tumors had been described earlier by SHRADER (I946), KIRKMAN (I962), KESTERSON and CARLTON (I970), and are extremely rare in relation to the cortex tumors.

Marrow-hyperplasias of the suprarenal gland or tumors, as STAEMMLER (I935) and ERMINKO (I955) observed them after chronic nicotine treatment in rats or cigarette smoke-condensate injections (MOHR and Assoc. I969), could not be proven by us.

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Source: https://www.industrydocuments.ucsf.edu/docs/mkk0000

III.
2 m Tumors of the Connective and Supportive Tissue

The observed tumors are compiled in chart 97 together with the tumors of the skin. The frequency of the sarcomas is unequivocally higher in the male animals.

The histological structure of the tumors (ill. 64 a - e) corresponds to the WHO classification for tumors in man. The total frequency of tumors is vastly in accord with former observations on the spontaneous frequency of sarcomas in the hamster:

FORTNER and GALE (I958)	among 620 = 6
FORTNER (I96I)	I8I = 2
KIRKMAN (I962)	7200 = 5
SICHUK and Assoc. (I966)	79 = 2
DUNHAM and HERROLD (I962)	360 = I
ASHBEL (I945)	I000 = 2
POPP, PREDETEANU (I960)	500 = I

Varied sarcomas of the connective and supportive tissues were also described by BUSCH (I953), CRABB and KELSALL (I954), KLEIN (I96I), LEE, TOTH and SHUBIK (I963), GRAUBMANN (I967), PATTERSON (I963), RUFFOLO and KIRKMAN (I965), GARCIA and Assoc. (I96I), FRIEDELL and Assoc. (I960), DELLA PORTA (I962), WEAVER (I952), LINDT (I958) GODGLUCK (I953).

Various sarcomas of the subcutaneous tissue were produced through local application of DEBA, (CHAUDHRY and Assoc. (I96I), CHAUDHRY and Assoc. (I96I), CHAUDHRY and Assoc. (I966), LEVY and RING (I950), LEE and Assoc. (I9 63), HOMBURGER and HSUEH (I970), RIVIERE and Assoc. (I963)).

Among the benign tumors, we would like to report on an odotoma (ill. 64 f), as a particularly remarkable case of a tumor, which had formed in the interior of the jaw-bone and showed epithelial and mesenchymal components. The tumor showed solid substances, respectively differentiation products in the sense of future tooth components.

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

III.
2 n Malignant New Formations of the Hematopoietic, respectively the Lymphoreticular Tissue.

To the spontaneous tumors of the goldhamster belong the tumors of the hematogenic and/or lymphoreticular tissue as some of the most frequently observed. FORTNER (I957 and I958) found among 620 hamsters I9 lymphosarcomas, STRAULI and HAMMERLI (I960) among 50 hamsters 5 lymphosarcomas, FORTNER (I96I) among I8I hamsters 5 reticulo cell sarcoma and I plasmocytoma, KIRKMAN (I962) among 7200 hamsters 2 reticulosarcomas and I plasmocytoma, STRAULI (I962) among 500 hamsters 32 reticulosarcomas, DUNHAM and HERROLD (I962) among 360 hamsters I2 reticulosarcomas, 3 plasmocytomas and 4 "lymphocytic tumors", HORN and SIEWERT (I968) among 760 hamsters 5 lymphosarcomas, respectively among I630 hamsters 30 lymphosarcomas. Malignant lymphomas were found by TOMATIS and Assoc. (I96I) (2 among 40 hamsters) as well as TOTH (I967) (9 among 200 hamsters). In a compilation by SHUBIK and Assoc. (I962), the frequency of malignant lymphomas in the controls is listed as I.3 % (= 549 animals). Besides that, individual observations of reticulosarcomas, plasmocytomas or lymphosarcomas were reported(FORTNER and GALE I95S, HANDLER and Assoc. I960, BRINDLEY and BANFIELD I96I, GARCIA and Assoc. I96I, LUCAS I96I, and RIVIERE and Assoc. I96I).

Conspicuous is the rare observation of leukemias. One case each were described by LINDT (I958), KIRKMAN (I962) and by HORN and SIEWERT (I968). Lymphatic leukemias were described in the hamster by GRAFFI (I97I) after infection of Papova-Virus.

After chronic treatment with DMBA, RAPPAPORT and Assoc. (I96I) saw in I of 24 hamsters, LEE and Assoc. (I963) in one of 53 hamsters and TOTH (I969) in I7 of 60, respectively 8 of 59 hamsters, malignant lymphomas. In a compilation by SHUBIK and Assoc. (I962), the frequency of malignant lymphomas, upon DMBA-treatment of the skin, is listed with 2.9 % (= I02 animals), while after oral treatment with 2-acetaminofluorene I.I %, with urethane 7.4 %, with 20-methylcholanthrene 7.0 % and with O-aminoazotoluene 6.7 % of the hamsters showed malignant new formations.

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Source: https://www.industrydocuments.ucsf.edu/docs/nlkk0000

Somewhat confusing is the histological classification of the observed tumors which was undertaken by the individual authors under very varying points of view. We set up the following classification (ill. 65 a - h)* for the observed malignant new formations of the hematopoietic, respectively the lymphoreticular tissue:

	macroscopically	microscopically
Leukemias lymphatic:	enlargement of liver, spleen and lymph nodes	in the liver predominantly periportal infiltrates of small lymphoid cells. Diffuse infiltration, for instance, of the lymph nodes, spleen etc.
myelonic:	enlargement predominantly of the liver, to a lesser extent than of the lymph nodes and the spleen	in parts, extreme diffuse infiltration of the liver with stron dissoziation, in parts, dense infiltration of lymph nodes and bone-marrow. Positive chloracetate-esterase reaction.
Lymphosarcomas type I:	in parts, diffuse, in parts, nodular enlargement, predominantly of lymph nodes, liver, spleen, bone-marrow. Metastases also in kidney, skin, myocardium and other organs	medium-size strongly basophile cells. Negative chloracetate-esterase reaction, vastly uniform lymphoid cell type.
Type II:	as type I	medium-size, strongly basophile cells. Negative chloracetate-esterase reaction. In between embedded are small round cells, particularly in tumors with strong bone-marrow displacement, in the liver myelopoiesis foci with positive chloracetate esterase reaction.
Type III;	as type I	large-cellular tumor with strongly basophile cells which resemble reticulum cells. Uniform cell type without small round cells. No myelopoiesis, no chloracetate-esterase reaction. Histological structure largely comparable to the reticulosarcoma in man.

* We thank Prof. Dr. Lennert, Director of the Pathological Institute of the University of Kiel for his assistance in classifying the preparations.

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkf0000

	macroscopically	microscopically
Myelosarcoma	diffuse to nodular infiltration of the organs	in parts, tumor-nodes from large basophile cells with broad cytoplasma. Profusely latticed fibers. Tumor cells with several nuclei. In tumor-nodes, in liver and spleen, foci of myelopoiesis with segmental nuclei cells, which show,in parts,a positive, in parts, a negative chloracetat esterase reaction. In liver and spleen abundantly polymorphous megacaryocites.
Plasmocytoma	in parts, individual tumor-nodes, in parts, diffuse and nodular infiltration of the organs	nearly pure cultures of plasm cells with typical structure and, in parts, distinct, in parts, minor polymorphy. In the liver here and there copious plasmacells in the capillaries (plasmacell leukemia ?)

The difficulaties in the delimitation between lymphosarcoma reticulasarcoma and myelosarcoma shall not be discussed in detail in this context. However, we would like to point out that the general term lymphosarcoma does not exclude the possibility of a myelogenetic origin of individual tumors. The delimitation between tumor and leukemia was also often difficult since we did not have blood-counts [illegible], for the animals which, without exception, all died spontaneously. Therefore, we regarde for instance, extramedullary myelopoiesis in the liver with lymphosarcomas of type II as compensatory hematogenesis with strong marrow displacement through tumor cells.

In the comparison of the test results between the different animal groups, we summed up the total number of available malignant new formations of the hematopoietic, respectively the lymphoreticular tissue, that is, leukemias and tumors were counted together, since a sharp differentiation between tumor and leukemia was often not possible, and because this summation was considered reasonable for the total evaluation of the test.

Source: https://www.industrydocuments.ucsf.edu/docs/mkkk0000

A correlation between the nature of the treatment of the test animals and the frequency of the appearance of tumors of the lymphoreticular and hematopoietic system is not recognizable (chart 98). However, in the animals of groups I and 2, treated with DMBA, a significantly earlier appearance of these changes is found, as compared to the controls as well as the animals only exposed to smoke.

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkf0000

3. Blood Tests after Smoke Treatment *

To supplement the morphological findings in smoke-exposed and not smoke-exposed gold-hamsters, we plan to discuss in the following, on hand of the available data, the question of the influence of smoke-substances on different biochemical and hematological blood values. Particularly in the evaluation of vascular processes, but also in the interpretation of hematological findings, a knowledge of the influence of the smoke treatment on the hematogenesis, on enzyme systems and other factors seems significant.

An abundance of literature is available on the influence of smoke-substances on the metabolism in man. The results were summarized in the two reports by the SURGEON GENERAL I97I and I972 as well as in an address by SCHIEVELBEIN and EBERHARDT (I972). In this summary are included numerous reports concerned with the problem of the influence of smoke-substances on blood coagulation, whereby SCHIEVELBEIN and EBERHARDT (I972) state that " a statement on the influence of nicotine on coagulation can hardly be made at the present time".

Material and Methodology:

The tests were carried out according to the initially described methodology. We used hamsters from the breed Zucca (USA) and from the breed Coombehurst(CMH) (England). The animals were accepted into the test after the usual quarantine. The age of the animals at test begin and the pertaining test length are listed in the charts I00 - I33. A further condensation of the charts was not possible according to statistical consideration because

a) the animals came from different colonies
b) the test times in respect to duration and the season were varied
c) the cigarettes with the same tobacco mixture were not completely identical because of different pesticide additives. The smoke-treatment was carried out according to the initially described method. The blood tests were made at the same time of day in all test animals between I0 - 20 min. after finished smoke-treatment. Blood for the counting of leukocytes and erythrocytes etc. was taken from the retro-orbital plexus of veins, the remaining examinations were made with the total blood of the hamster. The blood-letting was done after light ether narcosis by severing the aorta in the area of the thorax. The examinations of erythrocyte numbers, leukocyte numbers and hemoglobin values, lasting to the 56 th life-week, were undertaken on chronically smoke-exposed animals of group 5, respectively on animals of the control group K which were selected at random. From these animals, blood was taken under light ether narcosis from the retro-orbital plexus of veins. After the extraction of blood, the animals were returned to their respective groups.

*We thank Prof. Dr. Leuschner, Laboratory for Pharmacology and Toxicology, Hamburg, for carrying out the analyses.

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkd0000

For the smoke-treatment we used, as a rule, the standard cigarettes 3. In a part of the test results, the tests had been made with cigarettes which contained an admixture of pesticides. We included the values for these animals in order to facilitate a better survey on a larger material. The results of the pesticide tests will be reported at another time, they show no influence on the blood findings. The basic tobacco mixture of these cigarettes was equivalent to the standard cigarette.

The methods for the conducted tests are compiled in chart 99. For all values, we established the average value as well as the standard deviation and listed the range. The listing of comparative literature enables the reader to recognize the dispersion of the values in individual testing methods which is considerably higher for the goldhamster than for other test animals (e.g. rats).

Results:

The results of the blood tests are compiled in the charts I00 - I34. The use of hamsters from different colonies with, in parts, differing ages of the animals as well as conducting the tests at differing times, particularly seasons, and with differing numbers of animals, does not permit to consider all animals of the 3 groups (smoke-exposed group, fresh air controls, untreated controls) mathematically together, since, statistical differences are, in parts, already existent within the 3 groups. With exception of the erythrocyte values, the leukocyte values and the hemoglobin values for which we conducted an extensive statistical evaluation, we therefore limited ourselves in respect to the remaining biochemical data and blood values to the computation of the average values, the standard deviations and the listing of the range.

The findings which we obtained on a large stock of animals essentially correspond to values found by other authors. In comparison to pathological values, as they have been found in certain disease conditions and organ changes, the average values, the standard deviations as well as the range rest within the normal area for smoke-exposed and not smoke-exposed animals. Values which deviate distinctly from the norm are mentioned separately in the discussion of the individual data.

a. Hemoglobin

LARSON and SILVETTE (1968) compiled the reports which were concerned with the influence of smoke-substances on hemoglobin (Hb). According to them, BLACKBURN and Assoc.(I960)

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Source: http://www.industrydocuments.ucsf.edu/docs/mlkk0000

reported that the Hb does indeed show a tendency to increase in smokers, that it is not significant though. The remaining literature data found by LARSON and SILVETTE (1968) do also not show an unequivocal increase of Hb in smokers in comparison to non smokers. Experimental tests with animals dealing with the problem of the influence of smoke-treatment on Hb are not known to us.

Chart 100 shows the obtained Hb-values for control animals and smoke-exposed animals. The animals of group 5, smoke-exposed for over one year in chronic smoke-treatment tests were statistically compared to the pertaining control groups K. The result of the variance analysis shows a strong concurrence with the result of the statistical calculation for the erythrocytes. Here too, factors became evident which influence the Hb-value in a significant manner:

a) the treatment whereby the smoke-exposed animals showed higher values ($p < 1\%$),

b) the sex of the animals whereby the male animals, independent of the treatment, showed higher hemoglobin values ($p < 1\%$),

c) of the interreactions, that between smoke-treatment and weeks is significant ($p < 1\%$), that is, the influence of the treatment varies according to different weeks. The total influence of the treatment is 0 at 20 weeks for the smoke-exposed animals as compared to the controls, it rises up to the 44th week and is smaller again at 56 weeks.

d) the interreaction between treatment and sex is still contingent on coincidence.

The obtained values (chart 101) show a vast concurrence with the numbers stated in literature. The present experiment shows an unequivocal influence of the smoke-treatment on the Hb-quantity only with a long test duration.

b. Erythrocytes

In earlier experiments (RÖCKERT and DONTENWILL 1970), we were concerned with the influence of high-dosed smoke-treatment on the number of erythrocytes in the hamster (Colony "Seeboth").
With prolonged smoke-treatment (over 100 days), we saw an increase of the erythrocytes from 5.4 to 6.9 millions. This increase became even greater after a smoke-treatment length of 143 days and reached a ratio of 5.5 : 7.9 millions. We attributed the increase of erythrocytes to the chronic CO-intoxication which is unavoidable in the chosen tes

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

arrangement for the chronic smoke-treatment. In earlier experiments (REICHZEH and Assoc. 1969), we dealt in detail with the strong increase of the CO-Hb in smoke-exposed hamsters. Through the interval-free, long lasting exposure to smoke, the CO-Hb level rises considerably stronger than this can be observed in the smoking habits of man, where each puff is followed by a greater interval. We have not been able to find other experimental tests with animals on the influence of chronic smoke-treatment on the number of erythrocytes.

Chart 102 shows, in the comparison between test animals and controls, the increase of the number of erythrocytes in the smoke-exposed animals, their strength is dependent on the duration of the smoke-treatment. The compensatory increase of the erythrocytes, produced by the chronic CO-intoxication, is only understandable through the test arrangement and the increase of CO-Hb which it causes. However, this compensatory increase of the erythrocytes also shows that the animals are exposed to a strong CO-influence during the smoke-treatment and that this factor therefore has an effect which is multiply greater than it is in man during smoking. The findings as well as the quantitative examinations of the smoke-intake allow for the conclusion that the animals absorb considerable quantities of smoke-substances in the chosen test arrangement. The animals of group 5, smoke-exposed for over one year in chronic smoke-treatment tests, where statistically compared to the pertaining control groups K. In a variance analysis of the tests, we found as factors which influence the number of erythrocytes in a significant manner:

a) the treatment, whereby the smoke-exposed animals showed higher values ($p < 1\%$),

b) the sex of the animals, whereby the male animals, independent from the treatment, showed increased numbers of erythrocytes ($p < 1\%$),

c) of the interreactions, that between smoke-treatment and weeks is significant ($p < 1\%$), that is, the influence of the treatment varies with varying numbers of weeks. The total influence of the treatment is 0 at 20 weeks for the smoke-exposed animals as compared to the controls, increases up to 44 weeks and is less at 56 weeks.

d) the interreaction between treatment and sex is still contingent on coincidence.

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Source: https://www.industrydocuments.ucsf.edu/docs/mkkd0000

The obtained values show a vast concurrence with the numbers stated in literature (chart I03).

c. Thrombocytes, Prothrombin Time, Hematocrit Value

It would lead too far to discuss all the reports which deal with the influence of smoke-substances on blood coagulation, the agglutination of thrombocytes, respectively the factors of blood coagulation.

In his condensation, MURPHY (I968) writes about the thrombocytes, the thrombosis and the coagulation : "it is possible that smoking shows an acute effect, the formal proof is so far still lacking". Extensive literature can be found by LARSON and SILVETTE (I968) as well as by SCHIEVELBEIN and EBERHARDT (I972). They are almost exclusively tests, respectively observations on man.

Chart I04 shows that the number of thrombocytes is not unequivocally changed by smoke-treatment. However, the larger dippersion and range of the thrombocyte numbers is conspicuous in the smoke-exposed animals.

To supplement this, we would like to point out that BIRNSTINGL and Assoc. (I97I) found after treatment of rabbits with CO (400 ppm), a strong decrease of the number of thrombocytes (before 57, 5I per cmm - afterwards 33, 46) while at the same time the adhesiveness decreased from 30.04 % to 24.08 %.

The prothrombin time shows no change between smoke-exposed animals and controls. An influence on this coagulation factor is not recognizable (chart I05).

As can be recognized in a comparative observation of the hematocrit value and the number of corpuscular blood components, the hematocrit value rises parallel to the increase of erythrocytes (chart I06).

d. Leukocytes

An influence of nicotine on the number of leukocytes was studied by various authors (see LARSON and SILVETTE I968). MINUTH (I959) saw in smoke-treated rabbits one leukopenia, one granulocytopenia as well as an increase of the eosinophile leukocytes. Earlier in comparative experiments, we (REICHEN and DONTENWILL I970) had not been able to detect an unequivocal increase of the leukocytes after chronic smoke-treatment.

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

In an epidemiological study, CORRE and Assoc. (I97I) found an increase of leukocytes in smokers as compared to non-smokers. The differential blood-count did not show any essential changes. ASANO and Assoc. (I962) could prove that, after the smoking of 5 cigarettes, the total number of leukocytes rises parallel to the increase of the adrenaline level in the urine. He designated this as a "stress"situation. (Name illegible, Tr. and CASTRICIANO (I968) were able to prove an effect on the leukocytes in the periphery after the inhalation of cigarette smoke. In context to the question of a triggering of allergic reactions through smoke substances, SCHOEN and PIZER (I964), SCHRODER (I967) and SAVEL (I970) reported casuistic findings. They observed changes of the leukocytes in allergies, respectively tried to study, by means of different testing methods, the significance of tobacco substances for changes in the blood-count. The available findings do not give a uniform picture.

In chart I07 are compiled the total leukocyte numbers for the controls and the smoke-exposed animals. Chart I07 does not show any unequivocal differences between smoke-exposed animals and controls. We find a minor decrease of the leukocyte number in the smoke-exposed animals which runs parallel to the decrease in body-weight of these animals. In one test on not smoke-treated animals with reduced feeding, we were able to prove, as chart I08 shows, that a reduction of the body-weight by 36 % within 4 weeks also results in a decrease of the leukocyte numbers by 35 %. Since reductions of the numbers of leukocytes have been described in man upon a strong reduction of the body-weight (hunger) (VILLINGER-KNERCH I957), we assume that this factor was also decisive in the present case. In a statistical analysis of the chronically smoke-exposed animals of group 5 with the pertaining not smoke-exposed control animals of group K, we can regard as significant ($p < 5$ %), the interreactions of treatment and sex and the joint interreaction of treatment, sex and weeks. The total effect of the treatment is the summ of these effects: it presents for the smoke-exposed animals compared to the control animals an increase for the male animals, a reduction for the female animals of the values; this effect is more strongly pronounced in the 20th and 56th week than in the 30th and 44th week. These, in parts, high fluctuations of the numbers of leukocytes is on the basis of our own examinations not at all explainable through the presence of infects; a possible influence could be exerted by seasonal factors. The differential blood-count of the leukocytes shows a relative prevalence of the lymphocytes, a finding which is equally established by all researchers. An unequivocal influence of the smoke-

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Source: https://www.industrydocuments.ucsf.edu/docs/nkkd0000

treatment on the differential blood-count is not recognizable (chart II0, II2, II4, II6, II8).

A comparison to the findings or other authors is offered by charts I09, III, II3, II5, II7, II9.

e. Reticulocytes

Tests on the influence of smoke-substances on the reticulocytes are not available. Chart I02 shows that the average values and standard deviations between the smoke-exposed animals and the control groups, as well as the range, rest within the normal area. The low reticulocyte numbers in 2 or 4 control groups (groups 38 and 39) can be caused by the low age of the animals and/or by the season. RATHS (1957), for instance, found low reticulocyte values in summer (chart I20).

f. Electrolyte, Total Protein

We purposely carried out the determination of the electrolyte as well as the determination of other biochemical factors (e.g. total protein) within in the first 6 months of the life of the animal. The reasons rest in the increasing frequency and severity of the amyloidosis of the kidney in the goldhamster. Since, with increasing amyloidosis and thereby caused nephrosis, electrolyte shifts and changes of the blood protein consistency are conceivable, the question of the total protein and the electrolyte determination seemed more significant to us with an intact kidney parenchyma . Charts I2I - I23 show the obtained values for total protein and electrolytes. The electrolyte values and the total protein show, with the animals treated with fresh air also included in the consideration, no significant difference between smoke-exposed and not smoke-exposed animals.

A comparison with the findings of other authors is shown in charts I22 and I23.

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g. Total Bilirubin

Source: https://www.industrydocuments.ucsf.edu/tobacco/

g. Total Bilirubin

In order to clarify the influence of smoking on the functions of the liver, we determined besides enzymes the total bilirubin in the serum. No unequivocal differences are found between smoke-exposed animals and controls. The minimally lower values of the groups 38 and 39 in the untreated control animals may be caused by the low age of the animals (chart I24).

h. Enzymes: alkaline phosphatase, glutamate-oxalacetate-transaminase (SGOT), glutamate-pyruvate-transaminase (SGPT).

No information can be found in literature on the influence of smoke-substances on these enzyme values in the blood serum. It is conspicuous that in respect to SGOT in the hamster, a distinct sex difference is provable in individual groups. As the chart I25, in which the values are listed separately for the sexes, shows clearly, the average values for the male animals are, independent of the treatment, approximately twice as high as those for the females. Differences between treated and not treated animals cannot be proven (chart 126). How strongly the SGOT-values can rise, for instance, with infections (entamoeba histolytica), is demonstrated by the findings of RAETHER and Assoc. (1967). The SGOT-values rose from 42.0 (measured at room-temperature) after infections to maximally 4540.0. SGOT-values which were determined at 35 °C in the auto-analyser, after the method applied by us, are found by OPIE and Assoc. (1964). They found values of II7 ± 27.

The SGPT-values and the values of the alkaline phosphatase show no differences between treated and untreated animals (charts I27 and I28).

i. Total Cholesterin, Free Cholesterin, Esterized Fatty Acids, Free Fatty Acids, Neutral Fats

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We have already refered to the significance of the lipoid metabolism for the formation

Source: https://www.industrydocuments.ucsf.edu/docs/mkk00000

of arteriosclerosis in the discussion of heart and circulatory diseases.

Numerous researchers have concerned themselves with the question of the influence of smoke-substances on the lipoid metabolism. A combination of these experiments is given by the reports I97I and I972 of the Surgeon General, as well as by the monography by LARSON and SILVETTE (I968) and by the report by SCHIEVELBEIN and EBERHARDT (I972). Th findings which were obtained in animal experiments are considerably more varied than the findings obtained in man. In man (SCHIEVELBEIN and EBERHARDT , I972), a significa raised cholestrin level was found in healthy smokers, respectively through the inhala of smoke-substances an increase of the free fatty acids.

We compiled the results of our tests in the charts I29 - I33.

Free cholesterin, esterized fatty acids, free fatty acids and neutral fats do not sho any differing values in the treated and untreated animals, under consideration of the varied arrangements. For the total cholesterin, an increase in the treated animals is not provable. Under consideration of the strong dispersion of the values, a decrease of the total cholesterin value in treated animals is not evident. How strongly the total cholesterin can rise, is demonstrated by feeding experiments (cholesterin) by SHIRUK and BEHER (I969) who saw an increase from I38 ± 6.4I to 35I ± 57.4 and by WELL and ERBSHOFF (I962) who saw, with the same test arrangement, an increase from I32 ± 7 to 495 ± 32.

Altogether, the present biochemical findings of the serum and the hematological data after acute, subacute and chronic high-dosed smoke-treatment, show, that unequivocal changes were provable only for the erythrocytes and the hemoglobin. Both show a statistically significant rise which was considered to be a consequence of the test conditions (chronic CO-effect). The decrease in leukocyte values, proven in two groups, was only evident in female animals; it is possibly the result of the simultaneous decrease in body-weight caused by the smoke-treatment.

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Source: https://www.industrydocuments.ucsf.edu/docs/mkb80000

4. Comparison of Doses

In order to facilitate a comparison between the active smoking of man and the smoke-treatment of test animals, particularly however, to make a relation between dose and effect possible, a few explanatory data shall be cited.

The present-day smoke-norm (Coresta Standard No. IO)which also corresponds to the German Industrial Norm (DIN IO 240 - I969), is based on the assumption, that the smoker in the average smokes with a puff-volume of 35 ml., a puff-length of 2 sec. and a puff-frequency of I puff/min.

The approximately 35 ml smoke taken in per puff into the oral cavity can be exhaled by the smoker with the next breath without any previous inhaling, or he inhales with a new breath of variable quantity and therefore depth this smoke-quantity into the "breathing ducts". The average volume per breath is 500 ml. The "breathing ducts" would thus contain a smoke/air mixture of 35 : 465 ml. Approximately I50 - 200 ml fall to the share of the dead-air space. With the following expiration a part of the smoke/air mixture, particularly from the dead-air space, is exhaled without having reached the bronchi or the alveoli. A part remains in the respiratory tract depending on the depth of inhalation. A near I00 % intake of the smoke-substances is therefore impossible.

In our tests, we are essentially interested in the substances of the particle-phase of the smoke. Contingent upon the smoking habits of man inhaling via the oral cavity, we can assume, that, despite full inhalation, only approximately half of the smoke-particles remain in the respiratory tract, since, as already discussed, a part is exhaled and another part is transported away by the lung clearance.

Since there is a pause of 58 sec. between each puff of 2 sec., we have a relatively favourable situation for the removal of absorbed smoke-particles through exhalation and lung clearance.

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Source: https://www.industrydocuments.ucsf.edu/docs/

For the animal the conditions in our test arrangement are totally different. The animal breathes 3 x daily, IO min each time, from a vacuum-free system interval-free smoke/air mixture with a mixture ratio of I : I5. After a few breaths the lung contains only an equivalent smoke/air mixture. Since always the same mixture is offered, there are no puff pauses and therefore no purifying exhalation or time intervals for lung clearance. Under physiological conditions, the hamster has a breathing frequency of 75 breaths/min. On the basis of our own experiments (RUECKER and DONTENWILL I967), the frequency increases within the smoke-treatment period. If we take as a basis I00 breaths per minute during the smoke-treatment, the hamster inhales in IO min. approximately I000 puffs smoke/air mixture with a vast succumbing of the lung clearance. Because of the different flowing conditions, in contrast to man, in the area of the upper respiratory tract of the goldhamster, the strongest deposition of smoke-particles per unit of area takes place in the larynx. This concentration is approximately 300 x as great as in the lung area(DONTENWILL and Assoc. I97I, CHEVALIER and DONTENWILL I972). Since the dose per area is decisive for the effect, these factors receive a great significance. Furthermore, it must be considered that in the region of the cilia-free larynx parts, a purification is less easily possible and that the pavement epithelium reacts possibly more sensitively than the bronchial or tracheal cylinder epithelium (DONTENWILL and MOHR I962 b).

The evaluation of all factors shows that it is near impossible to establish an exact dose comparison between man and the animal, but that the effective dose on the larynx of the animal certainly constitutes a multiple of the dose effective in man per unit of area in the respiratory tract.

These calculation do not touch on the comparison between two test groups with the same methodology which is intended to show which dose or cigarette modification has a stronger or weaker effect.

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Source: https://www.industrydocuments.ucsf.edu/docs/nkk0000

The factors which lead to the highly applicated smoke quantity in the animal, strengthen to a particular extent the resorption of compounds which are absorbed from the smoke on account of an active participation of the body. This fact must be pointed out separately. The circumstances shall be discussed in more detail on two special components of smoke.

Carbon monoxide (CO) on account of its great affinity ~~of~~ to hemoglobin is greatly favoured in its absorption from the smoke/air mixture which is permanently offered to the animals for breathing during IO min. This makes the CO-Hb-value of the smoke-exposed animals understandable which rests near the toxic dose (RECKZEH and DONTENWILL I970). The CO-Hb-level is probably after each smoke-treatment considerably higher for longer periods of time than in the strong smoker (DONTENWILL and Assoc. I966). In the hamster, we must therefore also expect changes in the course of the smoke-treatment experiment, as they have been found by THEODORE and Assoc. (I97I) in various animals with nothing but CO-inhalation. This is evident in the present experiments with the hamster on the values of the erythrocytes, the hemoglobin and the hematocrit.

Nicotine is also faster resorbed from the smoke than other substances like for instance hydrocarbons, and is quickly transported away (DONTENWILL and Assoc. I97I, ISAAC and RAND I969). This facilitates a further resorption and the lung clearance can only have a limited effectiveness for the nicotine. Moreover, nicotine can also be resorbed in the area of the cavity of the nose and the oral cavity. We must therefore assume that the animals take in a high quantity of nicotine.

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

IV. Discussion

The present experiments were based on the following set of questions:

I. Does the smoke-treatment method which we developed represent a reproducable biological test?

2. Which of the obtained findings are attributable to the smoke-treatment of the animals and can we establish a dose-effect correlation?

3. Are quantitative differences ascertainable between the results from the individual test groups?

4. Can we draw conclusions from the combined treatments with smoke and various carcinogenic substances which would point to a co-carcinogenic effect, respectively an over-additive - in the sense of a co-carcinogenic - effect?

5. Evaluation of the remaining obtained findings which do not show a correlation to the treatment.

I. Smoke-treatment experiments of the same extent have so far not been conducted so that a basis for comparisons was not available. The vastly standardized biological tests in the area of the skin and the subcutaneous tissue permit solely the evaluation of the effect of the particle-phase of the smoke, mostly only through the inclusion of a solvent. Aging processes of the smoke as well as possible alterations of the biological effect through lack of the gas-phase are as much of a handicap in the epicutan-test as the various reactions of the skin, respectively the respiration epithelium, depending on anatomical and functional data. The same holds true for a subcutaneous application of the smoke-condensate.

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A decisive criterion for the particularly good suitability of the testing method is also the fact that it allows for information on the effect of the total smoke, whereby

Source: https://www.industrydocuments.ucsf.edu/docs/mkk00000

the freshly offered smoke comes to an effect in a quality which is equivalent to the smoke inhaled by the smoker. This decisive advantage (in the skin-test, it is the consensated particle-phase of the smoke, mostly days old, which is being tested) gives a greater validity to the information from the inhalation test, as well as the fact, that the smoke becomes primarily effective on the same organs as in the smoker, and arrives in the organism through the same ways as it is the case in the smoker. The reproduceability of our test shows that the methodology developed by us represents a useful biological test. It was in the present test, despite the use of another animal breed, not only possible to obtain vastly similar findings as in the first test (DONTENWILL I970). It became evident that in two groups (groups 5 and I2) treated in approximately the same way, vastly similar findings appeared in the area of the larynx. The simultaneous asbestos treatment of group I2 is thereby negligible because of its dose and effect which were too small, as the comparison to group I3, treated solely with asbestos demonstrates.

2. As chief effects of the smoke-components must be assessed the changes in the larynx, the hemoglobin and in the numbers of erythrocytes, the change in the life-expectancy and changes in the body-weight as well as the increase of certain changes in the lung like "smoke-cells".

In most of the animals, smoke-treated over a longer period of time (approximately I year), changes are provable on the locations in the respiratory tract on which the strongest concentration of the smoke-components in the sense of a deposit takes place. While in the larynx of an overwhelming number of the 960 control animals and animals treated with fresh air, no changes appeared, or at least only minor metaplastic changes of the epithelium of stage I and in one animal of stage 2, we found in animals, treated over a longer period of time with the total smoke, considerable epithelium changes of the stages 3 to 5 and carcinomas (stage 6) in large numbers. In studying the charts it becomes easily evident that the differences between the controls and

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

the treated animals must be termed unequivocal. This fact alone makes a comparative evaluation possible. It is facilitated even more, and the validity of the results is increased, when we compare the individual stages, according to the experiences in experimental cancer research with evaluation of the histological structure, to the change of the skin. The stages 3 and 4 in their significance could be most closely compared to papillomas of the mouse-skin. Stage 5 should be included in the group precanceroses "in the narrower meaning" which the term "pseudoepitheliomatous leukoplakia" was intended to express (see Atlas of the Armed Forces Institute). Stage 5 would perhaps be evaluated differently in a part of its cases by individual researchers. In the schematic representation we tried to characterize the stages morphologically in order to stress, for instance, the difference between stage 5 and the carcinoma. Only those changes were termed beginning carcinomas (stage 6) (early invasive carcinoma) which showed an infiltrative growing process. The frequency of the stages 5 and 6 is best suitable, in our opinion, for a comparison of the biological effect between two different test groups. When we evaluate the frequency of stage 6 alone, that is, of the carcinomas, the comparison can be established in the simplest way.

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The observation of the temporal succession of the individual stages shows that, whenever the biological effect is the least, the stages 5 and 6 appear more rarely, the preliminary stages 2, 3 and 4 however, are observed more frequently. In the individual stages, particularly in 3 - 6, it is not only a case of gradual differences in the dignity, but also of epithelium changes developing in temporal sequence, in which dose and time factors as well as factors of the biological effectiveness determine decisively, which degrees of epithelium changes will appear after a certain treatment.

Of the numerous biochemical and hematological findings, the changes of the hemoglobin and the erythrocytes are of particular interest. They are, as we had reported already earlier (DONTENWILL 1970), probably contingent on the chronic, high-dosed CO-intoxication which, through the test arrangement in the smoke-treatment, is multiply higher than in

Source: https://www.industrydocuments.ucsf.edu/docs/mkk00000

the smoking of man. The leukocyte reduction, traceable in individual groups, we attempted to explain through the reduction in weight. Surprisingly, no changes of the lipoids occured, eventhough the hamster, as a literature survey demonstrates, reacts with cholesterin feeding just as stronly as other test animals. Since the present smoke-treatment experiments were conducted on large numbers of animals and at different times, these findings deserve a greater significance.

Consequences of the chronic smoke-treatment are, besides a reduced consumption of food, the distinct reduction of the body-weight increase. The longer survival period of the untreated controls in contrast to the "fresh air animals" shows as well as the evaluation of the testicle changes that the remaining in the tubes of the smoke-treatment apparatus represents a factor which has a varying effect on the individual measured values (e.g. survival period) and shows the strongest reaction in the area of the testicle weights. The findings, obtained in the comparison of the animals treated with fresh air and the untreated controls, show, how important it is, precisely in smoke-treatment testing, to have a "placebo group" which allows in the calculation of the results to recognize the influence of the "test arrangement".

The opinion, postulated by us earlier, that the "adenomatoid lesions" are cases of preliminary stages of carcinomas (CHEVALIER and DONTENWILL 1971), will not be maintained by us any longer. Apparently, these are instances of changes which can be triggered by various factors, but which are not comparable to preliminary stages of tumors.

We can say with certainty that the term "smoke cells" is not conclusive since these changes are also found in animals not treated with smoke. They must probably, like the "adenomatoid lesions", be regarded as unspecific reactions of the lung, since smoke-cells were found much more frequently in animals smoke-treated over a longer period of time.

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Source: https://www.industrydocuments.ucsf.edu/docs/mkf03000

We gave particular attention to the appearance of emphysema. It was possible through the applied fixation method to compare quantitatively the changes in the lung. The diagnose emphysema was microscopically determined only relatively rarely and not at all unequivocally more frequently in the smoke-exposed animals. Therefore we carried out quantitative measurements with two different methods in individual test groups, with the question whether lung changes which can be termed emphysema will occur more frequently after chronic smoke-treatment. This was not the case, eventhough it is possible to produce a lung emphysema in the hamster through certain methods. This raises the question whether the total dosis reaching the lung is not high enough, or whether the hamster is not inclined in the same way to the formation of emphysemas as are other breeds of animals or man.

The provable dose-effect-correlation is another factor which must be assessed as an indication of the usefulness of the method. The increase in the frequency of the changes and the increase of their degree of severity in the larynx in the groups 4 and 5 shows that dose-effect-correlations are existing. The fact, that with the highest dos (group 6) a further increase was no longer provable, may have the same reasons, as we had also assumed them in our 4th Report on the Drop-Testing of Mice (DONTENWILL and Assoc. 1972). It is possible that an over-dosing, through higher toxicity and through a stronger deposit of smoke-components per unit of area which is no longer effective, will not show any more increase in the biological effectiveness. We were able to show unequivocally in our tests on the skins of mice (DONTENWILL and ASSOC. 1972) that a too high concentration of the tobacco-smoke condensate solutions (per unit of area) will no longer lead to an increase of the biological effect in the region of the trea area.

3. The present experiment is concerned as a chronic toxicity examination with the effect of the total smoke, respectively of certain smoke-components of very varied cigarettes, the smoke of which has been altered in quantity and quality through filte

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

or additives, or whose manufacture from sheets has led to an alteration of the smoke-substances.

Of particular interest are therefore those test results which show an influence on the biological effect through an alteration of the product. Since the present experiment with the large number of animals and the initially mentioned factors has a relatively high validity of evidence, it appears important to point out that the risk reduction, in the sense of a reduced biological effect, which was evident in the Drop-Tests (DONTENWILL, I970 and DONTENWILL and Assoc. I972) can also be proven in the same way in the inhalation test. The smoke of a same number of cigarettes was offered to the animals for the purpose of a comparison. The biological effect in the area of the larynx was very varied, particularly in the evaluation of the severe epithelium changes (stage 5 and 6) or the carcinomas alone (stage 6).

Inhalation of the gas-phase components alone led, similarly as in the animals treated only with fresh air, to metaplastic changes of the larynx of stage I only in 9 of I60 animals. - The strongest reduction of the biological effect was shown by cigarettes with acetate-filter and Dico-filter, the used sheet-cigarettes with and without $NaNO_3$ - additive as well as by the cigarettes with the black mixture. According to these findings distinct ways of a risk reduction result solely from a reduction or qualitative alteration of the offered smoke-components with the use of filter in the sense of a dose-effect relation. These findings confirm observations by WYNDER and Assoc. (I970) and by BROSS (I968) in epidemiological examiantions on smokers who prefer filter cigarettes, and by AUERBACH and Assoc. (I970) in experiments on dogs. But the tests also show that the already previously mentioned idea of an increased nitrosamin formation in cigarettes with $NaNO_3$-admixture is not at all proven yet.

The reduction of the biological effect of smoke on the larynx, recognizable in the comparison between the individual groups with the same dosage (e.g. between group 5 = 2 x 30 cigarettes of the standard mixture and group I4 = 2 x 30 cigarettes of the

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

black mixture) is once again discussed in chart I34.

4. Particularly unequivocal is also the difference between the animals pre-treated with DMBA and the animals treated only with smoke. It becomes evident here that the present methodology is also suitable for the interpretation of a syncarcinogenic or cocarcinogenic effect. If it were only a case of a syncarcinogenic effect, the summs of the effects of DMBA alone = group 2 and of smoke alone = group 5 should add up, and the summary effect should become evident in group I. The frequency of the stages 5 and 6 in group I is higher than the frequency of these stages in the groups 2 and 5 together, which must be interpreted as an over-additive effect. How far a cocarcinogenic effect can be drawn upon as an explanation remains open.

The tests show unequivocally, in our opinion, that the smoke-components attack in the same places as the strongly cancerogenically effective hydrocarbon DMBA, as the schematic graph 66 shows. The conclusion could be drawn from this finding that the hydrocarbons of the smoke constitute an important factor in the triggering of the epithelial changes on the larynx, and that the effect of the hydrocarbons is strengthened through cocarcinogenic factors of the smoke in the same place. That the effect is to a considerable extent a cocarcinogenic effect is confirmed by the tests of WYNDER and HOFFMANN (1961), BOCK (1958, 1969), VAN DUUREN (1963), VAN DUUREN and Assoc. (1966 and 1971) and others, as well as by our own observations on the skins of mice (DONTENWILL and Assoc. 1972) which show that cancerogens contained in smoke are potentiated through cocarcinogenic factors.

The combined tests with asbestos and smoke did not yield a result since the applied asbestos quantity was apparently too small.

Interesting are the findings which were obtained from those groups treated simultaneously with smoke and diethyl-nitrosamin. We purposely did not conduct an initial treatment because it leads to too great a loss in animals through the formation of tracheal papillomas. We already pointed that out in 1964 (DONTENWILL). These former observations

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

have now been confirmed by the experiments of NEHNER and Assoc. (1971) who proved, upon initial treatment with nitrosamins, an extraordinarily high mortality through papilloma formation in the trachea.

The nitrosamin treatment did not increase the frequency of the larynx changes. An explanation for that is extraordinarily difficult. When we consider the hitherto existing test results with combinations of nitrosamins and hydrocarbons, we see in a test by KOCH-LIGETTI and Assoc. (1963) in the combination of both carcinogens no increase in the tumors found. In a report by CEBALLOS and HERRANZ (1970) on the other hand, the carcinogenic effect of methylcholanthrene was reduced through an additional nitrosamin treatment.

From the present experiment we can recognize only one thing unequivocally: additional nitrosamin treatment does not produce an increase in the larynx changes produced by smoke. We were able to establish unequivocally that with nitrosamin treatment papillary epithelium growths are produced in places, as they occur in the same manner with nitrosamin treatment alone, and that they differ unequivocally in localization and structure from the changes produced by smoke. The findings show that the two factors smoke and nitrosamin have different places of attack (see graph 66), and that they are therefore not able to influence each other in their effect on the same place. The conclusion could be drawn from these results that nitrosamin does not deserve the significance in smoke which individual authors have attributed to it (DRUCKREY and PREUSSMANN, 1962; KROLLER, 1967; NEURATH, 1969; NEURATH and Assoc. 1965; PABLER and KLUS, 1971; PREUSSMANN and EISENBRAND, 1972; RHOADES and JOHNSON, 1970, 1972; SERFONTEIN and LURTNER, 1966). This question, much discussed today, should be elucidated through further experiments. The answering of this question is complicated by the fact, that the apparently in smoke most strongly represented dimethyl-nitrosamin (DMBA) shows a carcinogenic effect solely in the liver of the hamster but not in the lung. Even in the liver we could not find tumors more frequently in the animal groups which were smoke-treated with cigarettes containing a high additive of $NaNO_3$. Since however, most other nitrosamins,

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suspected or discovered in smoke, are carcinogenic in the hamster, this result deserv a rather large significance.

The problems of the nitrosamins must once again be placed in the foreground in the comparison of the effect of individual smoke-qualities. Since several authors (DRUCKREY and PREUSSMANN, 1962, RHOADES and JOHNSON, 1972) are of the opinion that the admixture of $NaNO_3$ favours a nitrosamin formation in the smoke, either an increased effect on the larynx should appear with a high $NaNO_3$ admixture, in the place where it also appears through smoke alone, or in those places where the changes are found with nitrosamin treatment. This however, was not the case with the nitrate admixture. The hypothesis of a nitrosamin effect in smoke has not found affirmation through the results of these experiments.

5. Besides the changes resulting from the smoke-treatment and cited as proof for the usefulness of the method, we plan to discuss in the following the remaining findin which we obtained. Chart I35 offers a good survey. Among the lung changes, we should mention rarely appearing adenomas which do not occur unequivocally more frequently wi the smoke-treatment. A lung carcinoma occured only in one animal, treated simultaneou ly with DMBA and smoke. Also in the remaining tumors of the pharynx and the trachea, of the cavity of the nose and the oral cavity, a higher frequency appeared only in those animals which received DMBA in addition.

Interesting is the papilloma, carcinoma and ulcus frequency in the stomach of the test animals. These changes apparently occur in a number of animals spontaneously. A difference in the frequency is not ascertainable between the smoke-exposed animals and the controls. An extreme rise in the papilloma frequency was recognizable solely in the animals of groups I and 2, treated with DMBA. However, no difference exists between the two groups. This result permits the conclusion that a carcinogenic or cocarcinogenic effect after smoke-treatment does not occur in the stomach.

The testicle changes appearing in the hamster in the sense of a testicle atrophy are

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

in parts, age-dependent, in parts, apparently caused through mechanical factors of the test arrangement. The comparative examinations of the testicle weights of smoke-exposed animals, animals treated with fresh air and untreated control animals show that the greatest loss in testicle weight is caused by the test arrangement, since fresh-air animals demonstrate a considerable reduction in the testicle weight already after a treatment of three months. The multiple transfers of the animals into the smoke tubes apparently produces a mechanical alteration and displacement of the testicles which are very sensitive to mechanical and thermic factors, as the literature survey shows. The somewhat stronger loss in testicle weight in the smoke-exposed animals could be expression of an additional influence. According to the entire findings, the hamster is not suitable for the evaluation of influences of smoke-components on the testicle tissue.

The frequently observed amyloidosis in the hamster, particularly the variously severe amyloidosis of the kidney to the point of the shrunken kidney shows no influence of the smoke-treatment. The amyloidosis is essentially age-dependent, as described already earlier by DONTENWILL and Assoc. (1960). In the severe amyloidoses, stage III, a weight increase of the heart is above all traceable in the untreated animals, which can be interpreted as a rise in blood-pressure through kidney shrinking. These changes however, do not lead to the pattern of an arteriosclerosis in the area of the aorta, the coronary arteries or the vessels of the leg.

The obtained heart changes, the changes on the valves as well as on the muscular system of the heart, are provable in smoke-exposed and not smoke-exposed animals. Also the findings on the vessels in the sense of an arteriitis (heart vessels and peripheral vessels) can be recognized in approximately the same frequency in the controls and the smoke-exposed animals. The present experiments do not show an etiologic influence of smoking on the formation of the described vessel processes.

The degeneration of the muscular systems of the skeleton and the heart which can be

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

observed in the hamster are not influenced by the smoke-treatment or the treatment with carcinogens.

The frequency of the tumors of the suprarenal gland, which belong to the most frequent spontaneous tumors in the hamster, does not show an influence of smoke-components.

The in all groups approximately equally frequent inflammable changes, particularly in the form of phlegmonous, subcutaneous inflammations as well as inflammable infiltration in the area of the head starting from the mucous membrane of the mouth, are not in any correlation to the smoke-treatment.

In a comparative observation of the remaining spontaneous tumors, carcinomas and sarcomas, e.g. carcinomas of the urinary bladder, tumors of the suprarenal gland and the liver as well as tumors of the lymphatic and reticular tissue, an unequivocal increas is neither provable in the smoke-exposed animals nor in the animals treated with carcinogens. That does not refer to the tumors of the oral cavity, the pharynx, the esophagus and omasum after DMBA-treatment as well as the papillomas in the lower larynx and in the trachea after nitrosamin-treatment. In the animals treated with DMBA, the tumors of the lymphatic tissue appear earlier or, in the earlier deceased animals, ar diagnosed earlier.

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Source: https://www.industrydocuments.ucsf.edu/docs/mlkk0000

V. Condensation

It was reported on a smoke-treatment experiment with cigarette-smoke, in parts, with simultaneous treatment with carcinogenic substances which embraced a total of 4.440 goldhamsters. 3.600 of these animals were treated over their entire lifetime, while the remaining 840 animals were smoke-treated up to 52 weeks.

The tests were processed and statistically evaluated with the aid of electronic data processing. In the test results were included survival period as well as body and organ weight development which were correlated, in parts, with the conducted treatment, respectively with the obtained morphological changes. Extensive patho-histological examinations of the organs and the tissue were carried out in order to seize all test-contingent changes.

Hematological as well as biochemical blood-tests were conducted on a large number of animals.

The present smoke-treatment experiment demonstrates that the developed method yields reproducable values in which a dose-effect correlation is provable. The method allows for a comparison of the effectiveness of the smoke from various cigarettes. The tests also enable an evaluation of combination treatments, e. g. smoke-treatment and treatment with carcinogens. A distinction was made in the obtained changes between:

I. Findings which were unequivocally produced by the smoke-treatment. Standing thereby in the foreground are larynx changes which we divided into 6 stages of severity. The appearance of larynx changes is dose-dependent. In a comparison between individual test groups distinct differences can be recognized. With respect to these changes it was possible to demonstrate unequivocally that certain test cigarettes (e. g. filter, cigarettes from a certain sheet or cigarettes with natrium-nitrate-additive) show an

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unequivocal reduction of the biological activity as measured on the larynx changes. This is particularly evident when the frequency of the most severe changes (stage 5 - pseudoepitheliomatous leukoplakia and stage 6 - carcinomas) is compared between the groups. The tests show furthermore that the appearance of the stages 2 - 6 is dependent on the length of the treatment and on the dose.

Upon combination of DMBA and smoke-treatment an over-additive effect becomes evident. While DMBA and smoke attack in the same localities, a simultaneous nitrosamin treatment demonstrated that smoke and DMNA show different points of attack in the respiratory tract. The significance of these findings is under discussion.

The smoke-treatment leads to a dose-dependent shortening of the survival period and to body-weight decrease.

The smoke-treatment caused an increase in the erythrocytes and in the hemoglobin. This finding is an expression of the high smoke-dosing contingent upon the test arrangement.

2. Findings which are unequivocally strengthened through the smoke-treatment. The so-called "smoke-cells", also traceable in the controls, are with high significance more frequent in the smoke-exposed animals, while the increase of the so-called"adenomatoid lesions" is only weakly ascertained in the smoke-exposed animals. The changes in the respiratory tract, triggered by DMBA,are over-additively intensified through the smoke-treatment.

3. Findings, triggered or intensified through DMBA, are: the increase in the tumors of the oral cavity, the palate, the pharynx, the esophagus, the stomach, the trachea, the liver and the ovaries and the appearance of ovarial cysts.

4. Findings, triggered by the nitrosamin-treatment, are papillomas of the lower larynx, which do not appear in the same manner in the smoke-exposed animals, as well as papillomas of the trachea.

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5. Findings which do not demonstrate any provable correlation to the treatment:

brain findings, tumors of the cavity of the nose, stomach ulcera (no dose-dependency), gastritis, lung emphysema, inflammations of the respiratory tract, generalized amyloidosis, testicle atrophy, vascular and heart diseases, muscular atrophy, formation of thrombi in heart and lung, cardiomyopathy, inflammable changes of the inguinal regions and the intestines, bile-duct cysts, bile-duct proliferation etc., Tumors of the skin, the connective and supportive tissue, the hematopoietic system and the suprarenal gland, hematological findings (except for erythrocytes and hemoglobin), biochemical findings.

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